THE RELATION BETWEEN BLOOD CHOLESTEROL LEVELS AND EEG CHANGES

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SUMMARY: Thirty three male albino rats, weighing 180-200 g were included in this experiment. Control animals consisting of 11 rats, were fed with a normal lab diet for a period of 12 weeks and the rest of the animals were fed with a diet containing 1% cholesterol for the same period. At the end of the experimental period, plasma cholesterol level (mean ± SD) was 134.04 ± 21.11 mg/dl in the cholesterol group and 72.72 ± 10.5 mg/dl in the control group. After feeding cholesterol group, eleven of the rats from the cholesterol group were transferred to the normal diet for 4 weeks (Normocholesterolemic group). The computerized spectral analysis of EEG records from parietal lobes of normal, hypercholesterolemic and normocholesterolemic rats by using the TRFC (Transient response-frequency characteristics) method showed that there was an obvious depression in the brain waves of hypercholesterolemic and normocholesterolemic animals.

Key Words: EEG, hypercholesterolemia, spectral analysis.

INTRODUCTION

Cholesterol/phospholipid, saturated/unsaturated fatty acids and lecitin/sphingomyelin ratios are the factors known to influence the fluidity of cell membrane (3,8,10,11). A close correlation on the other hand has been shown between the fluidity of the cell membrane and its excitability (1,4,13). It has furthermore been shown that the load of cholesterol have increased the frequency and amplitude of action potentials (4,13) have recently demonstrated that unsaturated fatty acids in central nervous system decrease the binding capacity of muscarinic receptors (12).

Membrane cholesterol and phospholipid levels are found to be a function of the composition of plasma cholesterol and phospholipids which inturn influence the permeability of the nerve membrane. These and other numerous *in-vitro* reports published in the literature (1,4), all indicate the relations between the cholesterol and neuron functions but no *in-vivo* studies exist concerning the same question. We have in our previous *in vivo* studies shown that (2,14) EEG is depressed and conduction velocity of afferent neurons are decreased by hypercholesterolemia.

We therefore investigated in a series of experiments on rats whether the depression in EEG produced by hypercholesterolemia would be alleviated after a period of hypocholesterolemia.

MATERILAS AND METHODS

Thirty three male albino rats, weighing 180-200 g were studied. They were divided into three equal groups. First control group was fed with a normal laboratory diet for 12 weeks; the other groups were given diet containing 1% cholesterol for the same period. In the end, first and second groups animals were deprived of food 24h and they were prepared for experimental procedure after ether anaetshesia. Disk electrodes were attached with collodion to the parietal and frontal positions along the midline (Pz and Fz, respectively) and to mastoid. EEG was recorded from the parietal region with reference to the mastoid. The grounding electrode was placed on the Fz.

EEG were recorded using San-ei 2G 66 amplifier and San-ei 5578 A poligraph. Amplifier gain was selected as 50-

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250μV/diV. Recording speed was 100 mm/sic. Blood samples were then taken by cardiac puncture. Aorta, brain and plasma samples were stored at -17°C until analysis were carried out. Total lipids (17), phopspolipids (7) and cholesterol levels (22) were measured in the plasma and in tissue extracts by Radin method (15).

The third group after feeding high cholesterol diet for 12 weeks was fed with a normal diet for 4 weeks. As a result, EEG were recorded and plasma cholesterol levels were measured as mentioned above.

The records analyzed were approximately 2 sec in duration. Digital amplitude measurements were taken every 10 msec totaling about 200 measurements for each record. EEG spectral analysis were computed at 0.5 Hz intervals from 1 to 50 Hz according to the method which is given in below.

Final elaboration of data was performed on the following frequency bands: 1-2 Hz, 2-4Hz, 4-6Hz, 6-8Hz, 16-32Hz, 32Hz and above.

Mathematical method

Amplitude-frequency characteristics or spectral analysis of EEG determine the frequency components and dynamic properties of brain structure in the frequency domain. Amplitude-frequency characteristics G(jω) were computed by using the Laplace transform (one sided Fourier transform) of the following form:

$$G(j\omega) = \frac{d}{dt}\int_0^{\infty} c(t)e^{-j\omega t}dt \quad (1)$$

G(jω) : Transfer function of the system.

c (t) : Step response of the system.

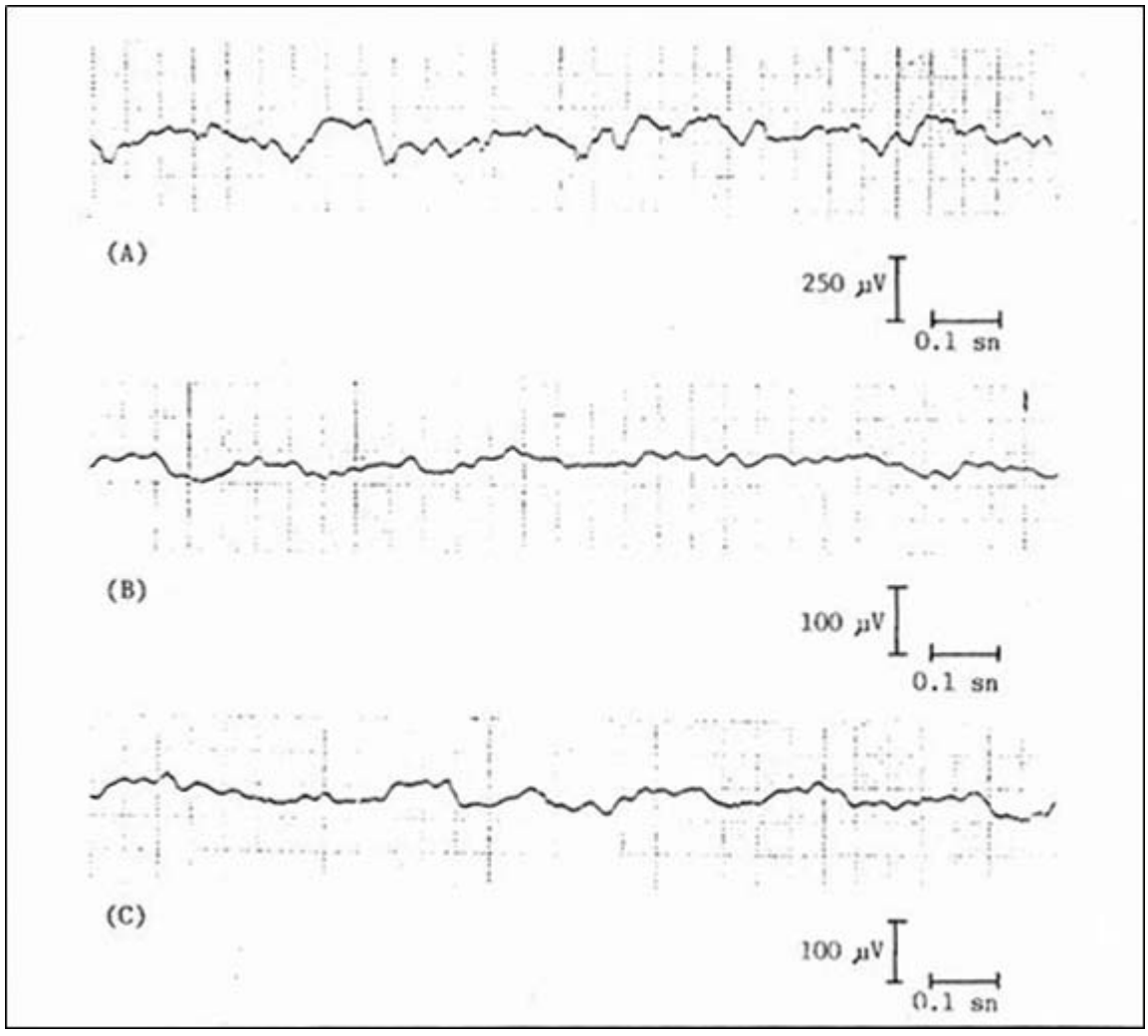
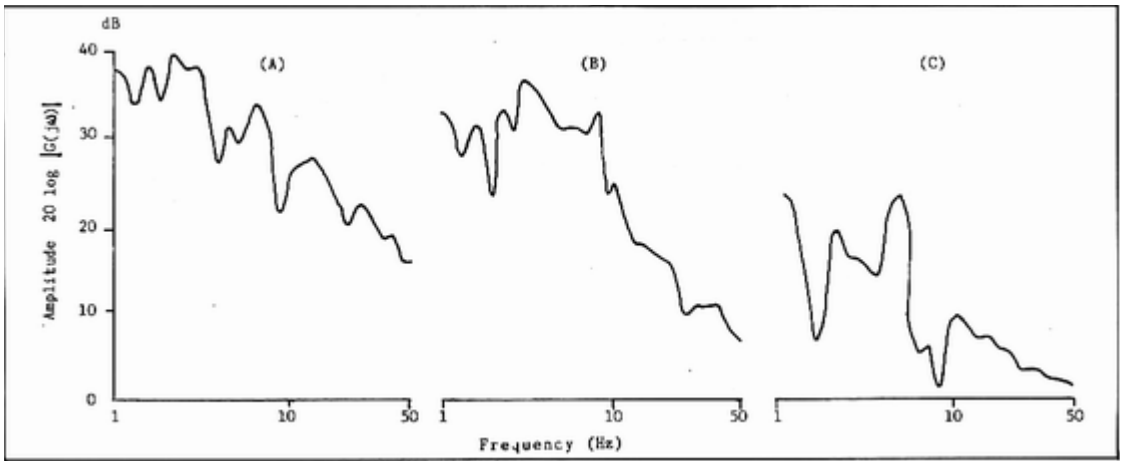


Figure 1: EEG activities recorded from partial lobes of three groups. (A) Control, (B) Hypercholesterolemic, (C) Normocholesterolemic

Figure 2: Amplitude frequency characteristics of three groups computed using EEG by TRFC method. Along the abcissa is the frequency in logaritmic scale, along the ordinate is the ampltude in decibels. (A) Control, (B) Hypercholesterolemic, (C) Normocholesterolemic



(Here EEG)

$\omega = 2\pi f$ f: frequency.

The amplitude frequency characteristics $|G(j\omega)|$ are obtained with the help of Ambtrad computer.

The use of the Fast Fourier algorithm for the evaluation of the integral (1) speeds the obtaining of frequency characteristics. Details concerning TRFC-method (Transient Response Frequency Characteristics method are given in references) (5,6,21).

RESULTS

From our results, it is possible to conclude that the increase in serum and aorta cholesterol levels of rats fed with cholesterol rich diet for a period of 12 weeks have showed the existence of hypercholesterolemia as shown in Table 1. After feeding of high cholesterol diet, eleven of the rats from cholesterol group were transferred to the normal diet for 4 weeks. The blood cholesterol was found to be 66.01 ± 13 mg/dl. This is the evidence that the animals became normocholesterolemia.

The electrical activities (EEG) of three groups of animals are presented in Figure 1.

Figure 2 represents amplitude-frequency characteristics computed from EEG using the mathematical method described (TRFC method) in "Materials and Methods". On the abscissa is the frequency in logarithmic scale and on the ordinate is the potential amplitude $|G(j\omega)|$, in relative units and decibels respectively. The curves are normalized in such a way that the amplitude at 0 Hz is equal to 1 or (20 log 1=0).

Maximal amplitudes are observed in some of the fre-

Table 1: Cholesterol and phospholipid levels of control and hypercholesterolemic groups.

	Control group (n = 12)	Hypercholesterolemic group (n = 12)
Serum cholesterol (mg/dl)	72.72 ± 10.5	134.04 ± 21:11*
Aorta cholesterol (mg/g wet wt)	2.50 ± 0.32	3.51 ± 0.26**
Cortex cholesterol (mg/g wet wt)	12.78 ± 0.65	13.50 ± 3.00
Brain cholesterol (mg/g wet wt)	16.73 ± 4.56	16.97 ± 5.35
Brain phospholipid (mg/g wet wt)	18.41 ± 5.96	17.67 ± 9.46
Cholesterol / Phospholipid	0.78 ± 0.16	0.97 ± 0.34

* P < 0.01, ** P < 0.05

quency bands as indicated. 1-2 Hz, 2-4 Hz, 4-6 Hz, 6-8 Hz, 8-16 Hz, 16-32 Hz, 32 Hz and above. The mean and standard deviation of the decibel (dB) values of the maxima in each frequency bands are seen in Table 2.

Comparing the hypercholesterolemic group (second group) with the control (first group), amplitude mean (dB) of 4-6 Hz frequency band is observed to have significantly decreased ($p<0.05$). Comparing the normocholesterolemic group (third group) with the control, the dB values of all frequency bands except 6-8 Hz, 16-32 frequency bands are also diminished significantly (Table 2).

When the number of animals displaying ampitude maximum in each frequency band was considered, it is found a significant result in (32 Hz and above) frequency band. It was observed that the percentage of the recordings obtaining the maximal amplitude were significantly different in three groups (Table 3). 90% of the tracings of the control animals revealed amplitudes reaching the maximal deflection while those in the hypercholesterolemia. This percentage remained at 54% and in the third group only at 40% of the cases. The difference between these means were highly significant ($p < 0.01$).

DISCUSSION

Our results of the control group computed by TRFC (Transient Response Frequency Characteristics) method are consistent with previous reported data (5,6,21). The power of EEG recorded from parietal lobes of second and third group animals was found to be decreased.

Our former experiments (14) have shown that depress on the brain activity is related to the increase in blood cholesterol level. Chemical analysis indicated that the brain cholesterol contents per gram of tissue in cholesterol fed animals was not significantly increased compared to the control group ($p>0.05$). EEG depression in the cholesterol fed group was however significantly depressed ($p< 0.01$). These reports (11,13,19) indicate that increased fatty acids reduce the binding capacity of insulin and of muscarinic receptors which may be a result of diminished permeability of the nerve membrane and reduction of electrical activity.

Schulte *et al.* (16) has reported that β waves are depressed in hypothyroidism and increased in hyperthyroidism. Other investigators (9) have claimed that unsaturated fatty acids are responsible of large amplitude waves observed in multiple sclerosis. The results of these inves-

Table 2: The mean and standard deviation of decibel (dB) values of maximum in each frequency bands.

Groups	1-2 Hz	2-4 Hz	4-6 Hz	6-8 Hz	8-16 Hz	16-32 Hz	32 Hz ve ↑
Control	39.68 ± 6.2	40.28 ± 6.42	36.89 ± 6.49	31.47 ± 8.91	29.47 ± 5.46	23.02 ± 5.5	20.22 ± 7.62
Group 1	34.66 ± 8.21 NS	35.89 ± 8.82 NS	29.76 ± 8.59 p < 0.05	29.95 ± 7.94 NS	25.55 ± 8.28 NS	17.28 ± 8.96 p < 0.05	16.62 ± 3.59 NS
Group 2	29.42 ± 6.68 p < 0.001	28.83 ± 7 p < 0.001	29.36 ± 6.97 p < 0.05	22.10 ± 9.61 NS	19.86 ± 9.42 p < 0.05	17.91 ± 9.7 NS	7.63±3.48 p < 0.01

Group 1: Hypercholesterolemic, Group 2: Normocholesterolemic, NS: Non-significant

Table 3: The percentage of animals displaying amplitude maximum in each frequency band.

Groups	1-2 Hz	2-4 Hz	4-6 Hz	6-8 Hz	8-16 Hz	16-32 Hz	32 Hz ve ↑
Control	%90	%82	%90	%72.7	%72.2	%90	%82
Group 1	%90	%90	%72.7	%82	%82	%82	%54.5
Group 2	%90	%82	%90	%82	%82	%82	%36.4

Group 1: Hypercholesterolemic, Group 2: Normocholesterolemic

tigations are in conformity with the conclusions obtained from our experiments.

In conclusion that the depressed EEG activity developed after hypercholesterolemia is not alleviated after one month of normocholesterolemia.

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