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Short communication

Essential fatty acid preparation improves biochemical and cognitive functions in experimental allergic encephalomyelitis rats

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Abstract

This study examined the possible effects of a novel mixture of fatty acids, SR-3 (a specific ratio of α-linolenic acids), on brain biochemistry and on learning deficits induced by injection of an agent that induces experimental allergic encephalomyelitis. Treatment with SR-3 caused a decrease in myelin and changes in the fatty acid profile of brain synaptosomes, and a learning deficit. Eighteen days of treatment with SR-3 reversed the biochemical and learning deficit significantly, but did not restore them to normal levels. We propose that, most probably, the main action of SR-3 is the modulation of the cholesterol level, which in turn causes the modulation of the fatty acid profile and enhances learning by allowing improved neuronal communication.

Keywords: Experimental allergic encephalomyelitis; Fatty acid; α-Linolenic acid; Linolenic acid; Multiple sclerosis

1. Introduction

Multiple sclerosis is characterized by active degradation of central nervous system myelin, with clinical symptoms dependent on the brain areas which undergo demyelination. The etiology of multiple sclerosis is unknown; however, one of the symptoms associated with multiple sclerosis is a deterioration of cognitive function (Ron and Feinstein, 1992). While an ideal animal model of multiple sclerosis is not currently available, experimental allergic encephalomyelitis is considered the preferred model (Werkele, 1993).

Relationships between multiple sclerosis and lipids and fatty acids have been proposed in the past (Swank and Grimsgaard, 1988; Williams and Deber, 1993), with changes in lipid metabolism being reported in the periphery of multiple sclerosis patients (Holman et al., 1989; Nightingale et al., 1990). In addition, changes in the level of cholesterol in the brain have also been described (Nicholas and Taylor, 1994). Unfortunately, attempts to alleviate multiple sclerosis symptoms with compounds containing $n-3$ and/or $n-6$ fatty acids have had only limited success (Field, 1989). A clarification of the role of particular fatty acids, such as linoleic and α-linolenic fatty acids (both are essential polyunsaturated fatty acids), may lead to a better understanding of the role of fatty acids in multiple sclerosis (Cunnane et al., 1989).

We have previously shown that the administration of a mixture of free fatty acids, α-linolenic and linoleic acids, in a ratio of 1:4 causes an improvement of cognitive functions such as maze learning in normal rats (Yehuda et al., 1994) and reverses learning deficits induced by iron deficiency or by neurotoxins (ethylcholine mustard aziridinium ion (AF64A) or by 5,7-dihydroxytryptamine). This mixture also has anti-seizure activity, improves thermoregulatory responses and raises the pain threshold in rats (Yehuda and Carasso, 1993; Yehuda et al., 1994, 1995). In addition, studies have shown that this mixture modifies the fatty acid profile of synaptosomes obtained from the frontal cortex of rats, resulting in an increase in the percentage of $n-3$ and $n-6$ fatty acids and a decrease in cholesterol level (Yehuda et al., 1996).

Because a decrease in myelin and an increase in cholesterol level have been reported in multiple sclerosis and

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because SR-3 has already been shown to change both, it seems reasonable to study the effects of this compound in a model of experimental allergic encephalomyelitis. The main methodological problem of using animals with experimental allergic encephalomyelitis is that the onset of the illness is very fast and the deterioration is swift. Experimental allergic encephalomyelitis animals, once ill, do not survive more than 2 weeks. We successfully replicated the method for inducing experimental allergic encephalomyelitis as demonstrated earlier by Nicholas and Taylor (1994). In addition, to enable measurement of behavioral variables, we administered a lower dose of the agent used to induce experimental allergic encephalomyelitis (L-experimental allergic encephalomyelitis) which created partially sick animals, i.e., rats with a reduction in the severity of symptoms. Rats that received a full dose of the agent inducing experimental allergic encephalomyelitis exhibited rapidly deteriorating motor activity with progressive ataxic motion, weaving, drunken gait and ineffective movement. In addition they had static tremor and shivering, did not react to a loud noise and, in the last stage, were unable to move. In comparison, the rats that received the L-experimental allergic encephalomyelitis dose also showed motor problems but these were much less severe. They exhibited drunken gait with ataxia, but the motion was effective. Shivering was much less severe and the animals reacted to a loud noise. In a preliminary study we determined that rats that received a full dose survived from 11–14 days, compared to rats that received the lower dose, which survived 26–27 days ($n = 10$ rats per group), a finding that confirmed earlier results by Nicholas and Taylor (1994).

The shivering observed in rats with experimental allergic encephalomyelitis may result from damage to the thermoregulatory control system. This is consistent with the reports of disturbances in body temperature among multiple sclerosis patients (Geny et al., 1992; Lammens et al., 1989).

A preliminary study was performed to determine the dose of SR-3 which significantly modified the effects of L-experimental allergic encephalomyelitis on the degree of myelination. Details are presented in Section 3. The lower dose of SR-3 (25 mg/kg) did not improve the degree of myelination. The higher doses of 40 mg/kg or 60 mg/kg did not differ from each other in improving the degree of myelination. Therefore, all the biochemical and behavioral studies were performed with rats receiving 40 mg/kg of SR-3. An earlier study (Yehuda and Carasso, 1993) showed that 3 weeks treatment with SR-3 is needed to established a clear effect on the learning capacity of rats.

This study investigated the effects of the fatty acid mixture SR-3 on (a) a spatial learning task (measured by the Morris water maze) and a passive avoidance task, (b) fatty acids profile and (c) cholesterol level. The latter two were measured by gas chromatography. The status of the myelin in the frontal cortex of treated and untreated rats was measured with the Luxol fast blue technique (Wakefield et al., 1994) and served as an index of the severity of the experimental allergic encephalomyelitis (full dose and lower dose) and the effectiveness of the SR-3 treatment. In this study we used the semiquantitative method of Yu et al. (1986) in which the amount of myelin is evaluated on a 5 point scale. In addition, body temperature was measured to assess the role of thermoregulation in the shivering observed in experimental allergic encephalomyelitis rats.

2. Materials and methods

2.1. Test material

α-Linolenic (0.92 g/ml) and linoleic (0.90 g/ml) free fatty acids, both 99% pure (as evaluated by capillary gas chromatography), were purchased from Sigma (St. Louis, MO, USA (L2367 and L1376)). The test substances were stored in the dark at 4°C. A fresh stock solution (1 ml) was prepared every 3 days by mixing 0.40 ml of α-linolenic and linoleic acid in a ratio of 1:4 in mineral oil (0.59 ml) and α-tocopherol (0.02 ml).

2.2. Animals

Male Lewis rats (250–270 g body weight) were used. They were housed individually in hanging, stainless steel, wire-mesh cages in a well-ventilated room that was air-conditioned by means of a system designed to maintain the room temperature at an average of 22°C and with a relative humidity of about 45%. The room was illuminated by fluorescent light that simulated the spectrum of the sun (Vita-Lite; Dura-Test; Clifton, NJ, USA) to permit an artificial 24-h cycle of 12 h of light daily (from 6 a.m. to 6 p.m.). Tap water and Altromine C-1000 diet were available ad libitum. The diet contained 5.1% fat.

2.3. Summary of experimental design

Each rat in the first experimental group ($n = 20$) was injected intradermally at 6 sites in both hind feet pads with a 0.25 ml suspension of 0.5 mg (wet weight) of whole guinea-pig spinal cord plus 3.0 mg of *M. tuberculosis* per ml of incomplete Freund's adjuvant (Nicholas and Taylor, 1994). Immediately after the experimental allergic encephalomyelitis treatment, 10 rats received SR-3 and 10 rats received mineral oil plus α-tocopherol. Subsequently they became very ill and exhibited flaccid paralysis of the hind limbs. The brains of these rats were analyzed by using the Luxol method. No behavioral studies were performed with these rats.

The second group of 60 rats received the experimental allergic encephalomyelitis inducing mixture, but the total dose was decreased to 0.12 ml (L-experimental allergic encephalomyelitis). Preliminary studies showed that at this

dose level rats survived 26–27 days. Twenty rats from this group received a daily injection of SR-3 for 3 weeks, 40 mg/kg i.p. Another 20 rats received an injection of mineral oil plus α-tocopherol and served as a control group, and the remaining 20 rats received saline. The third group of 60 rats received a saline injection in the hind pads and were divided into SR-3 treatment or mineral oil plus α-tocopherol treatment or saline ($n = 20$ per group).

In order to establish a baseline for the Luxol method, 10 rats received SR-3 and 10 rats received mineral oil treatment for 3 weeks, and their brains were analyzed for Luxol. On day 17 rats that received the L-experimental allergic encephalomyelitis dose were tested in an activity meter and their body temperature was measured. On day 18, after the beginning of treatment (SR-3 or mineral oil), all rats were tested in the Morris water maze for 3 days. On day 21 all were tested in the passive avoidance apparatus. On day 22 the rats were killed. For half the rats of each group the brain was analyzed for fatty acids and cholesterol, and for the other half for the Luxol fast blue study. The protocol was approved and supervised by the Animal Ethical Committee of Bar Ilan University.

2.4. Morris water maze

The Morris water tank (Brandeis et al., 1984) consists of a circular tank (110 cm in diameter) which was filled with water (to the level of 40 cm) and made opaque by the addition of powdered milk, so that rats swimming in the tank were unable to see an escape platform (7.5 cm in diameter) submerged 2 cm below water level. Each animal was released in the tank facing the wall in one of four predetermined starting locations, each separated by 90 cm around the inner perimeter. While the rat was in the tank, it was able to observe the contents of the room. Special care was given to keep things in the room in the same location. The rat could navigate in the tank only by external cues. Each rat was tested 8 times per day in the tank on 3 consecutive days. The order of the starting point was determined by random selection. Each rat was allowed 120 s to find the platform, with an interval of 20 s between trials. The maximum duration of the test for each rat was 16 min, and three rats were tested each hour. During this period, the platform was in the same location in the tank. For each of the 24 trials (eight trials on each of 3 days), the latency to reach the platform was recorded. A cutoff criterion, defined as the first successful trial with a maximum latency of 10 s without any increase in latency on a later trial, was used to calculate an index of learning for each group. In addition, the length of the swim path was recorded for each rat, as described elsewhere (Yehuda and Carasso, 1993).

2.5. Luxol staining for myelin

Rats were anaesthetized with sodium pentobarbital and their brains were removed en bloc. Each brain was fixed in 2% glutaraldehyde for 4 h and then transferred to a buffer solution for further processing. Paraffin-embedded tissue was cut at 6 μm and stain with Luxol fast blue. The dye Luxol fast blue was of the sulfonated copper phthalocyanine type. Two pathologists independently graded the degree of myelination semiquantitatively by microscope examination of the sections, with each having no knowledge either of the results obtained by the other or of the type of treatment the rat received. The agreement between the two pathologists was 97%. A grading system of 0–5 was designated as follows: 0, total absence of blue staining; 1, faint blue staining; up to 5, complete, confluent, blue staining (Yu et al., 1986).

2.6. Synaptosomes and determination of fatty acids and cholesterol

The level of fatty acids and cholesterol was determined by the gas chromatograph technique. Synaptosomes were prepared as suggested by Whittaker and Barker (1972). Brain tissues were homogenized on ice in 0.32 M sucrose, pH 7.0, and centrifuged at $23\,000 \times g$ for 20 min at 1°C. The supernatant was discarded and the pellet was resuspended in 6 ml of 0.32 M sucrose, applied to a discontinuous sucrose gradient (0.32, 0.8 and 1.2 M) and centrifuged at $100\,000 \times g$ (Model L8-55, Beckman, Palo Alto, CA, USA) for 60 min at 1°C. The synaptosomes were removed from the 0.8–1.2 M sucrose interface with Pasteur pipettes, diluted 1:1 with distilled water and centrifuged at $23\,000 \times g$ for 20 min at 1°C. The resulting pellet was resuspended in 1.0 ml of 0.32 M sucrose, rehomogenized and stored at −70°C until analyzed. Lipids were extracted from the membranes in a vial containing 15 ml chloroform/methanol (1:2, v/v) according to Folch-Pi et al. (1957). Recovery of synaptosomes was greater than 87% and the purity was determined by electron microscopy. Lipids were analyzed for fatty acid composition by gas chromatography (Varian, SP-2330 Supelco column, BPx70 Capillary column 50 m 0.33 mm ID, Model DB-23 SGE). The results from the gas chromatography were verified by mass spectrometry (4030 Finnigen-GS-MS, Sunnyvale, CA, USA). Cholesterol was analyzed with the same gas chromatography system, using a STIB-5 Supelco column, fused silica capillary column, 15 mm, 0.32 ID. Fatty acid quantification was done by comparison to GLC standard mixtures, GLC30-4-7040, AOCS-4-1019 and GLS60-4-7043 (Supelco, Bellefonte, PA, USA). The following variables were calculated: total fatty acids, fatty acid ratio (the ratio between saturated and unsaturated fatty acids) and cholesterol level.

2.7. Measurement of motor activity

The level of motor activity was assessed in an open field apparatus (75 cm × 75 cm) by recording the number of horizontal infrared photobeam crossings and rearing movements (determined from videotaped recording) which

were made during the 15 min sessions (Riekkinen et al., 1993).

2.8. Passive avoidance

The passive avoidance box consisted of a bright and a dark compartment. During the training trial (day 20) the rats were placed in the bright compartment. After the rats entered the dark compartment a shock was delivered (0.5 mA, 3 s). Twenty-four hours later the rats were again placed in the light compartment and the latency to re-enter the dark compartment was measured (maximum duration: 360 s). Short entry latencies indicated poor avoidance learning.

2.9. Body weight

Rats were weighed on day 0 and day 17.

2.10. Body temperature

Body temperature was measured by a telethermometer (YSI Telethermometer, Model 43TA, Yellow Spring, OH, USA).

2.11. Statistical analysis

There was no difference between the mineral oil and saline groups. Therefore, all statistical analyses were performed between the SR-3 group and the mineral oil group. All results are expressed as means with standard deviations (S.D.). The statistical significance of the mean differences was determined by analysis of variances (ANOVA) and Student's *t*-test.

3. Results

The 10 rats that received the full dose of the agent used to induce experimental allergic encephalomyelitis induction survived 11.4 ± 1.3 days. When SR-3 was administered to experimental allergic encephalomyelitis rats, they survived 17.1 ± 1.1 days although the severity of the symptoms was unchanged ($t_{(18)} = 10.718,\ P < 0.01$). When the lower dose of the experimental allergic encephalomyelitis inducing agent was administered to 10 rats that did not receive any treatment except for food and water, they survived 24.5 ± 1.3 days. Animals treated with SR-3 survived 29.3 ± 1.2 days ($t_{(18)} = 5.286,\ P < 0.01$).

The L-experimental allergic encephalomyelitis rats were lighter. The average body weight gain during the first 17 days was 2.0 ± 0.4 g for the group which received mineral oil plus α-tocopherol and 2.7 ± 0.6 g for the group which received SR-3 ($t_{(18)} = 3.1,\ P < 0.01$). For animals that received the saline injection the average body weight gain was 3.1 ± 0.9 g and 3.0 ± 0.8 g for SR-3 treated rats.

SR-3 was able to improve some of the learning deficits which occurred after the L-experimental allergic encephalomyelitis treatment. The effect of L-experimental allergic encephalomyelitis was evaluated and confirmed by Luxol fast blue staining. Control rats had a myelination score for the frontal cortex of 4.9 (± 0.31 point). Control rats which received SR-3 had a score of 4.9 ± 0.01. Rats treated with the full experimental allergic encephalomyelitis dose and which received mineral oil had a score of 0.4 ± 0.1 and those receiving SR-3 had a score of 0.7 ± 0.15. L-experimental allergic encephalomyelitis rats which received mineral oil had a score of 1.9 (± 0.73) and SR-3 treatment of this group increased the myelination score to 3.4 ± 0.51 (two-way ANOVA: main effect L-experimental allergic encephalomyelitis ($F(1,36) = 344.2,\ P < 0.001$); main effect SR-3 treatment ($F(1,36) = 74.2,\ P < 0.001$); interaction ($F(1,36) = 63.22,\ P < 0.001$)). Scheffe's analysis showed that the L-experimental allergic encephalomyelitis treatment group that received SR-3 was significantly different from all the other experimental groups ($\alpha = 0.05$). Furthermore, a highly significant *t*-test between the means of L-experimental allergic encephalomyelitis groups with and without SR-3 treatment was found ($t_{(18)} = 8.78,\ P < 0.001$). While the SR-3 restorative effects following a full dose of experimental allergic encephalomyelitis inducing agent may appear modest, at the reduced dose, as used in this experiment, SR-3 was able to dramatically improve behavioral performance, although there was no full recovery to the normal (pre-treatment) level.

The degree of myelination in the L-experimental allergic encephalomyelitis rats that received a dose of 25 mg/kg of SR-3 was 2.2 ± 0.95 and 1.7 ± 0.85 for the group that received mineral oil. The results of both groups were not statistically significantly different from those of the untreated L-experimental allergic encephalomyelitis group. The results for the 60 mg/kg group were 3.1 ± 0.81 for the SR-3 treated group and 2.0 ± 0.99 for the mineral oil treated group. These results were not statistically significantly different from those of the group receiving 40 mg/kg. Accordingly, we tested further variables using only the 40 mg/kg group.

Only L-experimental allergic encephalomyelitis treated rats and their control groups were tested in the behavioral tests. Rats treated with the full dose of the experimental allergic encephalomyelitis inducing agent were not tested. For every behavioral variable a 2-way ANOVA was calculated. All interactions were found to be significant.

Results similar to the biochemical findings were obtained for the learning data. The L-experimental allergic encephalomyelitis rats showed severe learning deficits. While SR-3 was able to reduce the number of trials for normal rats to reach criterion by one-third, i.e., from 18 to 6 ($t_{(18)} = 9.5,\ P < 0.001$), among the L-experimental allergic encephalomyelitis rats the reduction in the number of trials was from about 22 to 15 ($t_{(18)} = 4.32,\ P < 0.001$)

Table 1
Behavioral effects of L-experimental allergic encephalomyelitis treatment

	Control		L-Experimental allergic encephalomyelitis	
	Mineral oil	SR-3	Mineral oil	SR-3
No. of trials to reach criterion	18.5 ± 3.1	6.1 ± 2.5	22.1 ± 3.0	15.8 ± 3.5
Swimming span (cm)	500 ± 35.4	370 ± 45.3	1 232 ± 50.4	810 ± 63.1
Passive avoidance (max. 360 s)	325 ± 35	353 ± 6	100 ± 20	280 ± 25.6
Line crossing	750 ± 30	733 ± 26.3	427 ± 40	610 ± 45
Rearing	65 ± 5	68 ± 7	20 ± 3	24 ± 5
Body temperature	36.8 ± 0.7	37.1 ± 0.8	33.1 ± 0.5	35.9 ± 0.8

Effects of SR-3 on the learning of experimental allergic encephalomyelitis and L-experimental allergic encephalomyelitis rats, as measured by Morris water maze and passive avoidance, and changes in body temperature. See text for significant statistical differences.

(see Table 1). The swimming distance was also reduced following the SR-3 treatment: for the control rats from 500 cm to 370 cm ($t_{(18)} = 7.15,\ P < 0.001$) and for L-experimental allergic encephalomyelitis rats from 1232 cm to 810 cm ($t_{(18)} = 16.6,\ P < 0.001$). However, the L-experimental allergic encephalomyelitis rats that did not receive SR-3 covered a much longer distance (2.5 times longer) (see Table 1). While the SR-3 treatment did not improve the passive avoidance learning in the control rats, the treatment had significant effects in the L-experimental allergic encephalomyelitis rats, who improved from 100 to 280 s ($t_{(18)} = 17.8,\ P < 0.001$) but did not reach normal levels (Table 1). No correlation was found between body weight and learning. The SR-3 treatment did not affect motor activity in the control rats, but did improve the impaired motor activity of the L-experimental allergic encephalomyelitis rats, in that the line crossing was increased from 427 to 610 ($t_{(18)} = 9.61,\ P < 0.01$) and the rearing from 20 to 24 ($t_{(18)} = 2.16,\ P < 0.05$) (see Table 1). It should be remembered that (a) despite their motor problems the L-experimental allergic encephalomyelitis rats swam a greater distance and that (b) passive avoidance learning does not require motor activity. Experimental allergic encephalomyelitis rats exhibited hypothermia, with body temperature being 33.1°C compared to 36.8°C for the control rats ($t_{(18)} = 14.3,\ P < 0.001$) and SR-3 increased the body temperature to 35.9°C ($t_{(18)} = 9.39,\ P < 0.001$), only slightly below the normal temperature (see Table 1).

The profile of fatty acids of the L-experimental allergic encephalomyelitis rats was significantly different from the profile of the control rats ($P < 0.01$). There was an increase in the level of the 16:0 fatty acid, and there was a decrease in the level of 18:2($n-6$), 18:3($n-3$), 20:3($n-6$) and 20:4($n-6$) ($P < 0.01$). The treatment with SR-3 corrected these changes ($P < 0.05$). The fatty acid profile of L-experimental allergic encephalomyelitis rats differed from that of normal rats, mostly notably in a reduction in total fatty acids in the synaptosome (2.5 to 1.8, $P < 0.01$),

Table 2
Fatty acid composition of frontal cortex synaptosomes from rats with experimental allergic encephalomyelitis with or without SR-3 treatment

	Control		L-Experimental allergic encephalomyelitis	
	Mineral oil	SR-3	Mineral oil	SR-3
14:0	1.5 ± 0.6	1.3 ± 0.5	1.8 ± 0.9	1.2 ± 0.6
16:0	21.5 ± 2.6	21.9 ± 2.68	23.9 ± 2.8	21.6 ± 2.8
18:0	26.4 ± 0.9	22.1 ± 1.4	27.1 ± 1.3	25.3 ± 1.8
18:1($n-9$)	24.5 ± 1.9	25.9 ± 2.2	26.1 ± 1.8	24.8 ± 2.0
18:2($n-6$)	0.9 ± 0.4	1.9 ± 0.6	0.5 ± 0.4	1.2 ± 0.5
18:3($n-3$)	1.2 ± 0.5	3.7 ± 1.8	0.6 ± 0.5	1.0 ± 0.4
20:0	0.2 ± 0.1	0.04 ± 0.01	0.2 ± 0.1	0.1 ± 0.1
20:3($n-6$)	3.0 ± 0.8	2.5 ± 0.8	2.0 ± 0.5	2.2 ± 0.6
20:4($n-6$)	4.2 ± 1.3	3.1 ± 1.3	3.3 ± 1.4	3.5 ± 1.8
21:0	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	0.3 ± 0.1
22:1	2.5 ± 0.7	2.0 ± 1.0	2.9 ± 1.2	2.4 ± 1.5
22:4($n-6$)	2.6 ± 0.4	2.0 ± 1.5	1.6 ± 0.7	1.8 ± 1.0
22:6($n-3$)	11.1 ± 1.0	14.0 ± 1.7	9.2 ± 0.8	14.5 ± 1.2
Total fatty acids	2.5 ± 1.0	3.5 ± 1.4	1.8 ± 0.9	2.2 ± 0.8
Ratio S/US	1.10	0.87	1.30	1.03
Cholesterol	6.8 ± 2.1	4.4 ± 1.6	8.6 ± 2.0	6.9 ± 1.4

Effects of SR-3 on the profile of free fatty acids in synaptosomes prepared from the frontal cortex control and L-experimental allergic encephalomyelitis rats. Values are expressed as percentages of total fatty acid composition and are given as means ± S.D ($n = 10$). The total fatty acid content is expressed as a percentage of frontal cortex weight. Cholesterol is expressed as promille of frontal cortex weight. See text for significant statistical differences. S/US = saturated/unsaturated.

an increase in the ratio of saturated/unsaturated fatty acids (1.1 to 1.3, $P < 0.05$) and a significant increase in cholesterol level (6.8 to 8.6, $P < 0.01$). Treatment with SR-3 was able to modify the abnormal profile (see Table 2).

4. Discussion

The results concerning the effect of SR-3 on the fatty acid profile and cholesterol level in both the control and treatment groups are consistent with our previous study (Yehuda and Carasso, 1993). The changes in the cholesterol level are of particular interest. The importance of studying the cholesterol level follows from the work of Holman et al. (1989) who claimed that there is a decrease in the 'membrane fluidity index' among multiple sclerosis sufferers. Since cholesterol in the membrane decreases the fluidity index of the membrane, our finding of a significant increase in the cholesterol level would support this view. However, the role of cholesterol in multiple sclerosis is not well studied but the few studies on this topic indicate that there is an increase in brain cholesterol (Nicholas and Taylor, 1994; Syndyk and Awerbuch, 1994). The increase in the brain cholesterol level might result from two different mechanisms: (1) either an overproduction of cholesterol in the brain, or (2) low density lipoprotein (LDL), which is the major carrier of plasma cholesterol, may enter the brain in multiple sclerosis due to modification of the blood–brain barrier (Newcomb et al., 1994). Modifications of the structure and functions of the blood–brain barrier in multiple sclerosis patients have been reported before (Kwon and Prineas, 1994; Poser, 1994).

Lipids and fatty acids are major components of myelin. Therefore, changes in fatty acid metabolism or in the bioavailability of fatty acids and lipids in the brain will modify the composition of myelin and of the neuronal membrane. It is interesting that in 1983 Dhopeshwarkar noted that a deficiency in $n-3$ fatty acids is associated with an increased susceptibility to experimental allergic encephalomyelitis such that a smaller dose of the antigen was required to induce this disease (Dhopeshwarkar, 1983).

The possible link between the fatty acid profile and cholesterol level on learning and cognitive performance may be found in the optimal level of neuronal membrane function, expressed as a 'membrane fluidity' index. An optimal index allows the exchange of ions between the inside and outside of the membrane. This process is crucial for the transfer of neuronal information and for the proper activity of membrane receptors. Cholesterol produces rigidity of the membrane, whereas essential fatty acids increase the fluidity index. Experimental evidence supporting this hypothesis is summarized elsewhere (Yehuda and Carasso, 1993; Yehuda et al., 1994, 1995).

The Morris water maze and the passive avoidance task are two behavioral tasks which allow selected assessment of learning. They are widely used in evaluating learning deficits induced by brain lesions and pharmacological agents, as well as for evaluating new 'learning enhancer' drugs (Cunnane et al., 1989; Riekkinen et al., 1993; Yehuda and Carasso, 1993; Riekkinen and Riekkinen, 1994). It is clear, however, that these methods examine different aspects of learning. While the Morris water maze tests spatial capacity, the passive avoidance task reflects response inhibition in the presence of noxious stimuli. It is interesting to note that both types of abilities were deficient in animals treated with the lower dose of experimental allergic encephalomyelitis agent. These results may indicate that the effects of the experimental allergic encephalomyelitis are not restricted to one type of learning alone.

The results of this study indicated that SR-3 was able to repair the myelin damage following exposure to the experimental allergic encephalomyelitis antigen, to beneficially change the fatty acid profile, to lower the cholesterol level and to improve the learning performance. Changes in the myelin content and fatty acid profile of brain synaptosomes may interfere with the activity of ion channels and with the transfer of neuronal information, while changes in cholesterol may cause membrane hardening. Both mechanisms might help explain the deficits in learning.

The mode of action of SR-3 is still unknown. However, SR-3 has been shown to increase the level of free fatty acids in general and of polyunsaturated fatty acids in particular, and to decrease the level of cholesterol in synaptosomes. The associated improvement in learning capacity might be attributed to the normalization of the lipids in the brain.

Most investigations of the effects of essential fatty acids on animals with experimental allergic encephalomyelitis have studied the effects of γ-linoleic acid (which is derived from Evening Primrose oil or from a fungal source) on peripheral markers such as lymphocytes or red blood cells (Field, 1989; Phylactos et al., 1994). To our knowledge this report is the first study of α-linolenic effects on central variables (myelin and synaptosomal fatty acid profile). The results of this study together with those from previous studies (Yu et al., 1986) demonstrate that SR-3 has profound effects on brain biochemistry and on cognitive functions. This study also demonstrated that a lower dose of the agent used to induce experimental allergic encephalomyelitis is appropriate for the study of cognitive effects in this model. The changes in the level of myelin (as measured by Luxol), the changes in the fatty acid profile and the motor symptoms observed after treatment with the lower dose of the experimental allergic encephalomyelitis agent all contribute to the validity of this model as a faithful model of the early stages of multiple sclerosis, confirmed by the changes in the level of myelin which are comparable to changes observed in multiple sclerosis, and the changes in the fatty acid profile which are compatible to the results of Holman et al. (1989) concerning the profile of fatty acids in multiple sclerosis patients.

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