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Changes in the cholesterol level, cholesterol-to-phospholipid mole ratio, and membrane lipid microviscosity in rat brain induced by age and a plant oil mixture

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Among biochemical changes observed during aging, the composition of lipids in the brain exhibits major changes, e.g. an increased mole ratio of cholesterol to phospholipids and an increase in the degree of saturation of phospholipid acyl chains [1].

These and other changes contribute to a lowered membrane lipid fluidity and fluidity-related functions [2]. Several reports indicated that diet may affect membrane composition and function [3] and induce changes in longevity [4–6]. Rats fed with lecithin-enriched diets exhibit an elevated brain acetylcholine level [7] and influenced senescence-related disorders [8, 9]. A lipid mixture from egg yolk also lowers the membrane lipid microviscosity [10].

Recently, during studies on biological properties of a lipid mixture extracted from plants, we detected an oil fraction [containing, depending upon preparation procedures, 88–93% unsaturated fatty acids and coded Membrane Modulating Lipid (MML)] which modified *in vitro* the relative composition of plant protoplast membrane constituents (B. Kessler, to be published). In the present study we investigated *in vivo* effects of MML on the cholesterol and phospholipid levels in rat brain and their membrane lipid microviscosities.

Six male Sprague–Dawley rats at the age of 6 months (Weizmann Institute, Rehovot) were housed, one per cage, in a ventilated room at ambient temperature (22 ± 1°). The rats were fed an enriched diet with 3% MML. MML was dissolved in methylene dichloride from which a 3% MML-enriched diet was prepared. The animals were fed for 5 months throughout which the animals had free access to food and water. At the end of the 5-month period, the rats were decapitated, and their brains were removed and dissected into the hypothalamus, hippocampus, and cortex. A fourth part contained the brain stem and all remaining brain regions. All brain parts were quickly weighed and then gently ground with a Teflon homogenizer in an ice-cold 0.32 M sucrose solution, pH 7.4, at a ratio of 1:10 (w/v). The homogenates were centrifuged at 800 *g* for 10 min at 4°. The crude supernatant fraction which contained a mixture of cells and cell clusters, as determined by electron microscopy, gave highly reproducible results and was used for all the following assays.

Lipids were extracted from the supernatant fraction with chloroform–methanol (2:1, v/v). Cholesterol (C) was determined according to Abell *et al.* [11] and phospholipids after Bartlett [12]. Proteins were assayed in the aqueous fraction according to Sedmak and Grossberg [13]. Membrane lipid microviscosity was assayed by fluorescence depolarization employing the lipid probe 1,6-diphenyl-1,3,5-hexatriene (Fluka A.G.) according to Shinitzky and Henkart [2]. The lipid microviscosity was calculated from the empirical relation [14]:

$$\bar{\eta} = \frac{(I_\parallel/I_\perp) - 1}{0.73 - 0.27 \times I_\parallel/I_\perp}$$

Table 1 shows that feeding the rats with an MML-

Table 1. *In vivo* effects of MML on the level of cholesterol, the C/PL mole ratio, and the lipid microviscosity ($\bar{\eta}$) in rat brain*

Brain region	Age of animal and type of treatment		
	Young control	Old control	Old MML
	(1) Cholesterol (μg/mg protein)		
Hypothalamus	189 ± 48††	253 ± 11†	177 ± 4¶
Hippocampus	136 ± 23††	226 ± 40‡	171 ± 18**
Cortex	188 ± 33††	276 ± 22‡	257 ± 28‖
Stem and rest	275 ± 100††	396 ± 49†	350 ± 20‖
	(2) C/PL mole ratio		
Hypothalamus	0.37 ± 0.02‡‡	0.54 ± 0.03§	0.54 ± 0.06‖
Hippocampus	0.32 ± 0.05§§	0.60 ± 0.05§	0.50 ± 0.01¶
Cortex	0.38 ± 0.03§§	0.60 ± 0.01§	0.50 ± 0.01**
Stem and rest	0.47 ± 0.06§§	0.67 ± 0.03‡	0.67 ± 0.01‖
	(3) $\bar{\eta}$ (Poise, 22°)		
Hypothalamus	5.13 ± 0.31††	5.65 ± 0.02†	5.24 ± 0.02**
Hippocampus	4.71 ± 0.17‡‡	5.65 ± 0.004§	5.29 ± 0.09¶
Cortex	4.89 ± 0.11‡‡	5.50 ± 0.09§	5.20 ± 0.02¶
Stem and rest	5.50 ± 0.25‡‡	6.11 ± 0.09‡	6.05 ± 0.09‖

* Data represent mean ± S.E.M. The statistical analysis was carried out by Student's *t*-test.
† Non-significant, old control compared to young control.
‡ $P \leq 0.05$, old control compared to young control.
§ $P \leq 0.01$, old control compared to young control.
‖ Non-significant, old MML compared to old control.
¶ $P \leq 0.01$, old MML compared to old control.
** $P \leq 0.005$, old MML compared to old control.
†† Non-significant, young control compared to old MML.
‡‡ $P \leq 0.05$, young control compared to old MML.
§§ $P \leq 0.01$, young control compared to old MML.

enriched diet markedly influenced the levels of cholesterol, the C/PL mole ratio and $\bar{\eta}$. The cholesterol level in the MML-treated animals, compared to old control rats, decreased in the hypothalamus ($P \leq 0.01$) and the hippocampus ($P \leq 0.005$) and attained in all brain regions at least the levels found in the young controls. The C/PL mole ratio of the MML-fed rats was lowered significantly in the hippocampus ($P \leq 0.01$) and cortex ($P \leq 0.005$) as compared to the old controls but did not approach, except for the hypothalamus ($P \leq 0.05$), the mole ratio of the young animals.

The changes in cholesterol and the C/PL mole ratios were accompanied by appropriate changes in $\bar{\eta}$. MML, when compared to the old animals, led to a decrease of $\bar{\eta}$ in the hypothalamus ($P \leq 0.005$), hippocampus ($P \leq 0.01$), and cortex ($P \leq 0.01$), and reached values approaching those of the young control rats.

The above data strongly indicate that MML notably influences the composition of brain membranes (cholesterol and phospholipids), and modulates $\bar{\eta}$. We do not yet know the permeability of MML through the blood–brain barrier, but on the basis of its results on the above brain properties it is reasonable to assume that MML reaches the brain via the blood stream. We also do not yet know the mechanism(s) of the MML effects which may act by extracting cholesterol [15] (as indicated by the lowered cholesterol level) or by incorporation of MML into responsive membranes [16]. Potent effects by an active lipid mixture on $\bar{\eta}$ and the C/PL mole ratio have been reported in a number of papers [10]. The present work shows that pronounced modulating effects on cholesterol level, C/PL mole ratio, and $\bar{\eta}$ in at least the hippocampal and cortex regions of rat brain cell can be achieved by a diet with a lipid fraction prepared from plant sources. Detailed analytical studies of the various components of PL in the tissue samples and physiological and psychological implications of these findings are in progress.

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Biological activity of two novel inhibitors of uridine phosphorylase

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Inhibitors of pyrimidine nucleoside phosphorylases have been recognized as potential chemotherapeutic agents by virtue of their inhibition of pyrimidine salvage and by their modification of the metabolism of other chemotherapeutic agents [1–3]. The two mammalian pyrimidine nucleoside phosphorylases, uridine phosphorylase (UrdPase, EC 2.4.2.3) and thymidine phosphorylase (dThdPase, EC 2.3.2.4) share many common natural and synthetic substrates [1, 4], with a notable exception that uridine and 5-fluorouridine are substrates for UrdPase but not dThdPase [1]. Niedzwicki *et al.* [1, 5] reported the synthesis of several 5-substituted acyclic uridine derivatives which were more potent inhibitors of UrdPase than the corresponding uridine derivatives. Efficacy of these inhibitors is related to the hydrophobicity of the 5-substituent, for which a binding site exists on UrdPase [5, 6]. Thus, the solubility of these compounds is often quite poor in aqueous systems ([2] and Table 1). We report two new highly water-soluble acyclic nucleoside analogs with potent UrdPase inhibitory activity.

Materials and methods

5-Benzylacylcouridine (BAU),1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil (DHPBU), and (RS)-1-[[2-hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil (AHPBU) were synthesized in our laboratory.* [2-^{14}C]Uridine (56 mCi/mmole) and [2-^{14}C]thymidine (56 mCi/mmole) were purchased from Moravek Biochemicals, Brea, CA. UrdPase from S-180 cytosolic extract was prepared as previously described [1]. Human

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