Dolichol alters dynamic and static properties of mouse synaptosomal plasma membranes

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Dolichols are isoprenologues that are found in almost all tissues and whose biochemical function, aside from dolichol phosphate precursors, is not known. In addition, an understanding of the organizational and dynamic properties of dolichols in biological membranes has not been forthcoming. The purpose of the experiments reported here were to examine the effects of dolichol on the physical properties of mouse synaptic plasma membranes (SPM). Differential polarized phase fluorometry indicated that dolichol both fluidized and regidified SPM. Membrane areas detected by diphenylhexatriene and *trans*-parinaric acid were selectively fluidized and rigidified, respectively. It also was found that the spin label, 5-doxyl stearic acid indicated that dolichol reduced membrane fluidity. These results report for the first time a stuctural effect of dolichol on a biological membrane.

Dolichol *Fluorometry* *ESR* *Plasma membrane* *Isoprenolog*

1. INTRODUCTION

Free dolichol has been reported as a component of liver plasma membranes [1,2] and brain membranes [3]. Accumulation of free dolichol in human and mouse brain is associated with aging [3–6] and with neuronal degenerative disorders such as neuronal ceroid-lipofuscinosis [3] and Alzheimer's disease [7]. Several reports using electron spin resonance probes [8], fluorescence probes [9], differential scanning calorimetry [9,10], ^{31}P-NMR [10], freeze fracture electron microscopy [10] and X-ray scattering [10] indicate that free dolichol (0–1 mol%) dramatically fluidizes artificial model membranes and promotes formation of the nonbilayer hexagonal phase II form in phospholipids such as phosphatidylethanolamine. The purpose of the studies described here was to determine whether these findings are also relevant to a biological membrane, the synaptosomal plasma membrane (SPM). Differential polarized phase fluorometry indicated that membrane areas detected by diphenylhexatriene and *trans*-parinaric acid were selectively fluidized and rigidified, respectively, by dolichol. The fluorescent fatty acid data were in agreement with those using 5-doxyl stearic acid and ESR techniques. These results report for the first time a structural effect of dolichol on a biological membrane.

2. MATERIALS AND METHODS

Bovine serum albumin, fatty acid free (BSA) and pig liver dolichol (98%) were purchased from Boehringer Mannheim, Indianapolis, IN and Sigma, St. Louis, MO, respectively. 1,6-Diphenyl-1,3,5-hexatriene and *trans*-parinaric acid were from Aldrich, Milwaukee, WI and Molecular Probes, Junction City, OR, respectively. 5-Doxyl stearic acid was from Sigma.

Male C57BL/6NNIA (6 month old) mice were obtained from the National Institute on Aging

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Colony maintained by Charles River, Wilmington, MA. Mice were housed, fed, killed by decapitation, brains dissected, and synaptosomal plasma membranes prepared by a method [11] modified as described in [12]. Dolichol was incorporated into synaptosomal plasma membranes (SPM) by first binding with BSA as follows. Dolichol, 66 μg, was evaporated onto the bottom of an acid washed glass test tube. Then 250 μl of a 5% BSA solution in deionized water was added. The tube was degassed with N_2, sealed with teflon tape and a teflon cap, and placed in a 37°C shaking bath overnight. For the blank condition, a similar tube minus dolichol was set up. The BSA-dolichol or BSA solution was then transferred to a 10 ml polycarbonate centrifuge tube containing 250 μl (0.66 mg) SPM in phosphate-buffered saline (PBS), pH 7.2. This was then degassed, sealed with a teflon cap, and incubated with shaking at 37°C for 1 h. The sample was then diluted to 10 ml with PBS and sedimented at 40 000 × g for 20 min at 4°C with a 40 Ti rotor on an L565B ultracentrifuge (Beckman, Fullerton, CA). The pellet was resuspended with 10 ml PBS and again sedimented.

The quantity of dolichol actually incorporated into SPM by the above method was determined by HPLC [4] after lipid extraction by the method of Folch [13].

1,6-Diphenyl-1,3,5-hexatriene (DPH) and *trans*-parinaric acid were incorporated into SPM and all fluorescence parameters (lifetime, polarization, differential polarized phase fluorescence) were determined as described [14]. Limiting anisotropy and rotational relaxation time (ns) were obtained according to Lakowicz et al. [15], basically as described in [14]. The order parameter for DPH was evaluated as $(r_\infty/r_0)^{1/2}$ [16]. All electron spin resonance procedures were performed as described [12].

3. RESULTS AND DISCUSSION

Although free dolichol is known to fluidize artificial model phospholipid membranes, this effect has heretofore not been demonstrated in a biological membrane. The dolichol content of SPM from young (6 month old) mice, before and after exogenous dolichol incorporation, is 391 ± 45 and 3358 ± 258 ng/mg protein, respectively. Thus only 3.4% of the pig liver dolichol in the incubation mixture is actually incorporated into the SPM. The level of dolichol observed in the untreated SPM compares favorably with that found in plasma membranes of liver and brain (300–1800 ng/mg protein) reported by others [1–3]. Most important, after treatment with exogenous dolichol, the level of dolichol found in SPM from 6 month old mice resembles that in SPM from 24 month old mice of the same strain, 3954 ng/mg protein.

Dolichol significantly ($p<0.01$) reduces the limiting anisotropy and order parameter of DPH in SPMs (table 1). This observation indicates that the resistance of the lipid microenvironment surrounding DPH to the motion of DPH is reduced due to dolichol incorporation. A reduction of DPH limiting anisotropy of 0.009 (table 1) in SPM is equivalent to raising the temperature of the SPM from 37.0 to 39.5°C. This reduction in limiting anisotropy is also equivalent to adding an intoxicating acute dose of ethanol (100 mM) to the SPM [17]. Ethanol (160 mM) decreased the order parameter of a spin labeled fatty acid about 0.8% [18] while herein dolichol decreases the order parameter of DPH by 3.1%. In contrast to its effects on the structural or static properties of DPH in SPM, dolichol does not affect the rotational relaxation time of DPH. Thus, dolichol affects the static but not dynamic aspects of DPH motion. A similar observation has been made for the effect of temperature on DPH motion in model membrane vesicles [15].

In contrast to the results obtained with DPH, dolichol increases the limiting anisotropy and decreases the rotational relaxation time of the microenvironment occupied by *trans*-parinaric acid in the SPM (table 2). Thus, the resistance to *trans*-parinaric acid motion and the rate of rotation (the rate of rotation is equal to the reciprocal of 6 × rotational relaxation time) increase. This result is confirmed by electron spin resonance measurements using 5-doxyl stearic acid, another type of fatty acid probe molecule (table 2). Dolichol increases the order parameter or resistance to probe motion.

In these studies, two important observations were made. First, the effects of dolichol on the physical properties of a biological membrane, the SPM, were reported for the first time. Second, dolichol had a two-fold effect, one of which differed markedly from the results reported in ar-

Table 1

Effect of dolichol on dynamic and static properties of fluorescence probe molecules in synaptosomal plasma membranes[a]

Preincubation conditions	Limiting anisotropy r_∞	Rotational relaxation time $(6R)^{-1}$, ns	Order parameter S
1,6-Diphenyl-1,3,5-hexatriene			
BSA	0.190 ± 0.001	1.23 ± 0.03	0.700 ± 0.003
BSA-dolichol	0.181 ± 0.001*	1.26 ± 0.09	0.678 ± 0.003*
Trans-Parinaric acid[b]			
BSA	0.161 ± 0.002	2.26 ± 0.14	–
BSA-dolichol	0.188 ± 0.003*	1.50 ± 0.06*	–

[a] Synaptosomal plasma membranes were isolated from mouse brain and probe molecules were incorporated as described in section 2. Values represent the mean ± SE ($n=3$). An asterisk refers to $p<0.01$ by Student's t-test as compared to BSA pretreatment alone

[b] Values for limiting anisotropy and rotational relaxation time of *trans*-parinaric acid refer to average values calculated from an intensity weighted average lifetime

tificial model membranes. Namely, dolichol fluidized the lipid microenvironment of SPM sensed by DPH in a similar fashion as previously reported by a variety of techniques in membranes composed of artificial phospholipids [9,10]. In contrast, free dolichol was not reported to rigidify any of these model membranes. Therefore, observation that dolichol increases the rigidity of the SPM lipid microenvironment surrounding the fatty acid probes (both fluorescence and ESR) demonstrates additional structural complexity in the biological membrane not reported in the artificial membrane systems.

Table 2

Effect of dolichol on electron spin resonance properties of 5-doxyl stearic acid in synaptosomal plasma membranes[a]

Preincubation conditions	Probe molecule	Order parameter S
BSA	5-doxyl stearic acid	0.5958 ± 0.045
BSA-dolichol	5-doxyl stearic acid	0.6156 ± 0.0039*

[a] All conditions were the same as described in the legend to table 1 except that 5-doxyl stearic acid and dolichol were concomitantly incorporated into the synaptosomal plasma membrane followed by sedimentation and washing. Values represent the mean ± SE ($n=4$). An asterisk refers to $p<0.01$ by Student's t-test

The dolichol content in brain is known to increase dramatically with age [1,4–6] and in diseases such as Alzheimers [1] and ceroid-lipofuscinosis [1,19]. These changes may have differential effects on the membrane structure and on the lipid microenvironments of the SPM. Future investigations will clarify whether individual SPM functions may also be differentially regulated by dolichol, depending on the microenvironment wherein they reside.

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