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The influence of dolichols on fluidity of mouse synaptic plasma membranes

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Dolichols are isoprenologues which constitute an important component of biological membranes. However, an understanding of the effects of dolichols on the organization and dynamics of biological membranes has not been forthcoming. The experiments reported here are aimed at understanding the effects of dolichols on the physical properties of mouse brain synaptic plasma membranes. The effect of dolichols incorporated into mouse brain synaptic plasma membranes on fluorescent and electron spin resonance probes sensing the hydrophobic core differed from that of probes reporting closer to the surface of membrane bilayers. Dolichols significantly ($P < 0.01$) lowered the polarization, limiting anisotropy, and order parameter of diphenylhexatriene in synaptic plasma membranes and liposomes extracted from synaptic plasma membranes, without changing the rotational relaxation time. Similarly, dolichol increased the fluidity reported by 16-doxylstearic acid in synaptic plasma membranes or liposomes extracted from synaptic plasma membranes. In contrast, dolichols exerted no effect on those properties for *trans*-parinaric acid or 5-doxylstearic acid in synaptic plasma membranes or liposomes derived therefrom. Dolichols can dramatically alter the structure and dynamics of lipid motion in synaptic plasma membranes and these effects are dependent on the location of the probe in the membrane.

Introduction

Almost all tissues [1,2] and eukaryotic cell membranes [1,3–5] contain poly *cis*-isoprenoid lipids called dolichols Dolichols exist as free dolichol, dolichol phosphate, or fatty acyl dolichyl esters Free dolichols are present in plasma membranes of liver and brain [3–5], normally in quantities ranging between 0 3 and 1 8 μg/mg protein (approx dolichol/lipid mole ratio 0 2–1 0 1000) Dolichol content of both human and mouse brain increases as much as 10-fold with age, and large accumulation is found in Alzheimer's disease, and ceroid lipofuscinosis [3,6–8] Thus far, a function for only the phosphorylated dolichol form has been established, namely as a chemical 'carrier' of saccharide units during membrane directed assembly of glycoproteins [9] However, most investigations indicate that dolichol phosphate constitutes less than 10% of the total dolichol content [10] Little is known about the functions of the esterified dolichols in membrane or the free dolichols which generally comprise greater than 80% of total dolichols in membranes

Singularly lacking is an understanding of the

Abbreviations 5-NS, 5-doxylstearate, 16-NS, 16-doxylstearate

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organizational and dynamic parameters of free dolichol, as well as the dolichol phosphates and dolichol esters, in biological membranes Studies measuring 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization in phospholipid model membranes have reported that dolichol at 1 1000 molar ratio significantly increased the fluidity [11] and permeability [12] of phosphatidylethanolamine-containing membranes However, it is not known if the artificial membrane fluidization induced by extremely low quantities of dolichol [11] is relevant to a biological membrane In this manuscript we report the use of fluorescence and electron spin resonance (ESR) probe molecules to investigate effects of dolichol on a biological membrane, namely, the mouse brain synaptic plasma membrane

Part of this work was presented as an abstract Schroeder, F , Wood, G , Gorka, C , Strong, R , Sun, A Y and Sun, G Y (1986) Fed Proc 45, 752 (a3467)

Materials and Methods

Reagents Bovine serum albumin, fatty acid free was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN Pig liver dolichols (98%), 5-doxylstearate (5-NS), and 16-doxylstearate (16-NS) were from Sigma Chemical Co , St Louis, MO 1,6-Diphenyl-1,3,5-hexatriene and *trans*-parinaric acid were purchased from Aldrich Chemical Co , Inc , Milwaukee, WI and Molecular Probes, Junction City, OR, respectively

Animals Male C57BL/6NNIA (6 month old) mice were obtained from the National Institute on Aging Colony maintained by Charles River, Inc , Wilmington, MA Mice were housed for 1 week and fed commercial laboratory mouse chow (Ralston-Purina, St Louis, MO) prior to use

Synaptic plasma membrane and liposome preparation All animals were killed by decapitation, brains were dissected, and synaptic plasma membranes prepared by a method [13] modified as described elsewhere [14] Each preparation represents synaptic plasma membranes obtained from pooled brains of four animals The marker enzyme $(Na^+ + K^+)$-ATPase was purified 5–6-fold in synaptic plasma membranes with respect to crude homogenate For preparation of liposomes, synaptic plasma membranes were added to chloroform/methanol (2 1, v/v) and vortexed for 1 min [15] The sample was centrifuged at $1000 \times g$ for 10 min The bottom layer was removed and filtered through glass wool using anhydrous sodium sulfate The filtered sample was evaporated to dryness under a stream of nitrogen and brought up in chloroform and stored at $-20\,^\circ$C An aliquot of the lipid extract was evaporated to dryness and resuspended in 50 mM Tris-HCl (pH 7 4) and used as the liposome preparation Phospholipid was quantitated [16]

Fluorescent probe incorporation and fluorescence spectroscopy Synaptic plasma membranes (50 μg/ml phosphate-buffered saline (Ca^{2+} and Mg^{2+} free, pH 7 4) or liposomes were pretreated with buffer or dolichol Dolichol (10% of synaptic plasma membrane protein by weight) in CCl_4 was evaporated onto the bottom of an acid washed glass test tube Synaptic plasma membranes or liposomes in 50 mM Tris (pH 7 4) were added and incubated at 24° C for 1 h This procedure resulted in increasing dolichol content from 391 ± 45 ng/mg synaptic plasma membranes protein to 3358 ng/mg protein as determined by HPLC analysis The sample was then transferred to a new acid washed test tube and incubated under N_2 at 37° C for 20 min with 0 025 μg diphenylhexatriene in 0 5 μl tetrahydrofuran Every 5 minutes the sample was vortexed for 15 s. *Trans*-parinaric acid (2 μg) was dried with N_2 on the sides of a glass tube, followed by addition of sample as above, and incubated under N_2 at 37° C for 30 min

Fluorescence parameters were measured with two instruments Absorbance, absorption-corrected fluorescence, relative fluorescence efficiency, and corrected fluorescence emission were determined simultaneously with a computer centered spectrofluorimeter previously described [17,18] Light scatter (or turbidity) was reduced by using narrow band passes in the excitation and emission monochromator When light scattering was detectable, fluorescence intensities were corrected by subtracting the signal of an analogous synaptic plasma membrane preparation without added fluorescence probe molecules The scattering correction was always less than 2% Excitation wavelengths for diphenylhexatriene and *trans*-

parinarate were 358 and 313 nm, respectively, while emission was measured at 430 and 415 nm, respectively

All other fluorescence parameters (lifetime, polarization, differential polarized phase fluorescence) were determined as described earlier [19] Lifetime and differential polarized phase fluorescence were measured at 6, 18 and 30 MHz with an SLM 4800 Subnanosecond Fluorometer, interfaced to an IBM PC Computer Data were acquired and analyzed by Program ISS-O1 (ISS Instr, Champaign, IL) In this program the set of phase and modulation lifetime data was statistically analyzed by a nonlinear least-squares routine [20,21] The data were fitted to one- or two-exponential decay terms In the latter case, each term was characterized by a lifetime, τ, a fractional intensity, F, and a mole fraction, α The reduced Chi (x^2) parameter was used to judge quality of fit [21] Chi values near 3 were considered acceptable while values in excess of 10 were indicative of large discrepancies between calculated theoretical curves and the experimental data The error in each parameter was determined using a correlation matrix of errors [21]

Steady-state anisotropy, measured with the SLM 4800 in the T-format, was corrected for grating anisotropies [22] Steady-state anisotropy was also corrected for light scattering by use of appropriate cut-off filters In addition, samples were serially diluted and steady-state anisotropy was measured and then extrapolated to zero absorbance [23,24]

Rotational relaxation time (ns) and limiting anisotropy were obtained at 6, 18 and 30 MHz according to the method developed by Weber [25] and Lakowicz et al [26] as described earlier [19] The order parameter for diphenylhexatriene (based on $r_0 = 0\,392$, Lakowicz et al [26]) was evaluated as $(r_\infty/r_0)^{1/2}$ [27] Each value for limiting anisotropy, rotational relaxation time, and order parameter represents the mean of nine determinations on the same sample determined at 30, 18 and 6 MHz

Unless otherwise specified, all data were obtained at 37°C

Electron spin resonance spectroscopy Dolichol in CCl_4 (10% of synaptic plasma membrane protein by weight) and 5- or 16-doxylstearic acid were evaporated onto the bottom of an acid washed glass test tube Synaptic plasma membrane in 50 mM Tris buffer (pH 7 4) was added After incubation, the pellet was drawn up into a 100 μl micropipet and placed in a Varian E 109E EPR spectrometer (Varian Associates, Palo Alto, CA) and read at 37°C Spectra were recorded at X-band frequency using 100 kHz modulation The microwave power was 5 mW, modulation amplitude was 2 G, scan range was 100 G and microwave frequency was 9100 MHz for all samples The order parameter S and $2T'_\perp$ were calculated by the method of Hubbell and McConnell [28] For membranes labeled with the 16-doxylstearic acid label, only $2T'_\perp$ was calculated Each sample was scanned 4 to 5 times Liposomes in chloroform were evaporated to dryness and reconstituted with 50 mM Tris-HCl (pH 7 4) Procedures for spin label and dolichol incorporation were used as described above

Results

Effect of dolichol on spectral characteristics of 1,6-diphenyl-1,3,5-hexatriene in synaptic plasma membranes

Diphenylhexatriene is rapidly and maximally incorporated into synaptic plasma membranes within 15 min at 37°C Excitation and emission maxima of diphenylhexatriene in synaptic plasma membranes are obtained near 343, 358 and 376 nm (Fig 1A), and near 402, 425 and 449 nm (Fig 1B), respectively Dolichol does not alter the spectral shape or the location of the diphenylhexatriene excitation or emission maxima These results indicate that the diphenylhexatriene is located in a similar microenvironment before and after preincubation with dolichol

Dynamic and static properties of diphenylhexatriene in synaptic plasma membranes before and after pretreatment with dolichol

Nonlinear least-squares analysis of diphenylhexatriene phase and modulation lifetime data indicates that the fluorescence lifetime of diphenylhexatriene in synaptic plasma membranes is primarily comprised of one component, $\tau = 10\,5$ ns at 24°C, chi values less than 3 (Table I) Occasionally, a second component near 2 ± 3 ns comprising less than 3% of the total fluorescence

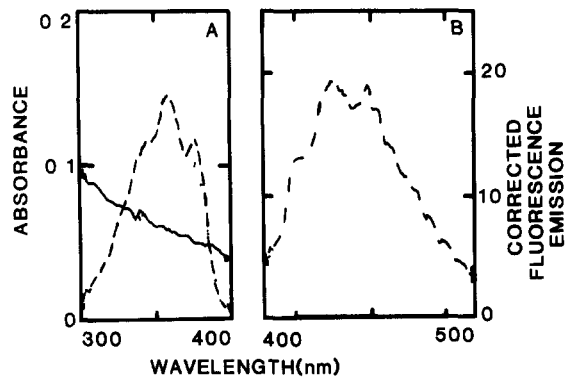

Fig 1 Spectral characteristics of 1,6-diphenyl-1,3,5-hexatriene in synaptic plasma membranes Synaptic plasma membranes (50 μg/ml phosphate-buffered saline, pH 7 4) were incubated with dolichol and 0 05 μg 1,6-diphenyl-1,3,5-hexatriene for 20 min at 37 ° C as described in Methods Absorbance (A, ———), absorbance-corrected fluorescence (CO, - - -), and relative fluorescence efficiency (RFE,), were determined simultaneously at 24 ° C with emission at 425 nm Absorbance-corrected fluorescence emission (CFE, - -) was determined at 24 ° C with excitation at 358 nm

intensity can be resolved (Table I) This short lifetime component represents an artifact that is insensitive to lipid structural alterations [29] In addition, with only three fixed modulation frequencies available with the SLM 4800 instrument, this component cannot be accurately and reproducibly resolved Therefore, the lifetime data are analyzed for fitness to single exponential decay and reported as such

In the past, most measurements of diphenylhexatriene dynamics in membranes have been made by the Perrin equation to obtain a rotational correlation time in nanoseconds [30] However, this measurement is actually comprised of both dynamic and static components [26] These two components of diphenylhexatriene motion in synaptic plasma membranes are resolved by differential polarized phase fluorometry, polarization, and lifetime determinations (Table I) The rotational relaxation time and limiting anisotropy of diphenylhexatriene in synaptic plasma membranes at 24 ° C are 1 19 ns and 0 232, respectively, indicating rapid but restricted motion of diphenylhexatriene in synaptic plasma membranes At 37 ° C, the restriction to motion, as indicated by the limiting anisotropy, decreased from 0 232 (at 24 ° C) to 0 185 ($P < 0\ 01$), without significant change in rotational relaxation time If the Perrin equation rotational correlation time $= \tau/[(r_0/r) - 1]$ alone, is utilized, a 66% decrease in diphenylhexatriene rotational correlation time at 37 ° C vs 24 ° C is obtained (11 0 vs 18 0 ns) Thus, from the Perrin equation it may be concluded that an increase in temperature drastically increases the dynamic properties of diphenylhexatriene motion in synaptic plasma membranes In contrast, when the static and dynamic properties of diphenylhexatriene motion in synaptic plasma membrane are resolved by differential polarized phase fluorometry (Table I) it is apparent that restriction to probe motion (or order) is decreased by increasing temperature while rotational rate, the dynamic term, is not significantly affected

Preincubation of synaptic plasma membranes or liposomes derived from synaptic plasma membranes with dolichol significantly decreased both the fluorescence polarization as well as the limiting anisotropy of diphenylhexatriene (Table II) These results indicate that dolichol decreases the restriction of lipids to diphenylhexatriene motion

Dolichol alters dynamic and static properties of trans-parinaric acid in synaptic plasma membranes

In contrast to results obtained with diphenyl-

TABLE I

DYNAMIC PROPERTIES OF DIPHENYLHEXATRIENE IN SYNAPTIC PLASMA MEMBRANES AT 24 ° C and 37 ° C

1,6-Diphenyl-1,3,5-hexatriene (0 05 μg) was incorporated into young mouse synaptic plasma membranes as described in Methods Polarization, lifetime (τ), limiting anisotropy (r_∞), rotational relaxation time [$(6R)^{-1}$], and order parameter (S) were determined at 24 ° C and 37 ° C as described Values represent the mean ± S E ($n = 3$), an asterisk and and double asterisk represent $P < 0\ 01$ and $P < 0\ 05$, respectively, between 37 ° C and 24 ° C values

Temp (° C)	Polarization	τ (ns)	r_∞	$(6R)^{-1}$) (ns)	S
24	0 3303 ± 0 0025	10 50 ± 0 16	0 2316 ± 0 0018	1 193 ± 0 066	0 7688 ± 0 0029
37	0 2826 ± 0 0011 *	9 74 ± 0 16 **	0 1854 ± 0 0010 **	1 200 ± 0 106	0 6874 ± 0 0022 **

TABLE II

EFFECT OF DOLICHOL ON DIPHENYLHEXATRIENE FLUORESCENCE IN SYNAPTIC PLASMA MEMBRANES OR LIPOSOMES EXTRACTED FROM SYNAPTIC PLASMA MEMBRANES

Values represent the mean ± S E ($m = 3$–5) An asterisk refers to $P < 0.05$ by Student's t-test as compared to (−) dolichol

Membranes from	Dolicol	Polarization	Limiting anisotropy
Synaptic plasma	−	0.285 ± 0.003	0.210 ± 0.002
Synaptic plasma	+	0.277 ± 0.004 *	0.198 ± 0.003 *
Liposomes	−	0.269 ± 0.004	0.188 ± 0.005
Liposomes	+	0.254 ± 0.002 *	0.160 ± 0.005' *

TABLE III

EFFECT OF DOLICHOL ON *TRANS*-PARINARIC ACID FLUORESCENCE IN SYNAPTIC PLASMA MEMBRANES AND LIPOSOMES

Values represent the mean ± S E ($m = 3$–5)

Membranes from	Dolichol	Polarization	Limiting anisotropy
Synaptic plasma	−	0.223 ± 0.002	0.184 ± 0.004
Synaptic plasma	+	0.222 ± 0.002	0.174 ± 0.005
Liposomes	−	0.192 ± 0.012	0.159 ± 0.006
Liposomes	+	0.204 ± 0.008	0.160 ± 0.004

hexatriene, preincubation of synaptic plasma membrane with dolichols does not affect the polarization or the limiting anisotropy of *trans*-parinaric acid in synaptic plasma membranes or liposomes derived therefrom (Table III) In addition, the dolichols do not change the rotational relaxation time (data not shown)

trans-Parinaric acid may partition preferentially into the solid vs fluid membrane lipid regions [31–33] Fluorescence lifetime analysis of *trans*-parinaric acid in synaptic plasma membranes indicates the presence of two lifetime components (Table IV) Statistical chi values for closeness of fit to two lifetimes are between 5 and 8 ($n = 3$) Attempting to fit the data to a single lifetime gives chi values in excess of 300 The appearance of two lifetime components near 18 3 and 2 0 ns at 37 °C can be interpreted as representing the lifetime of *trans*-parinaric acid in solid and fluid regions, respectively The mole fraction of *trans*-parinaric acid in these regions is 0 22 and 0 78, respectively In previous studies, it was shown that the preferential partitioning of *trans*-parinaric acid into solid vs fluid lipid phases is 2 9–3 3-fold [31–34] These data allow an estimation of the content of solid phase (or solid clusters) lipids in synaptic plasma membranes Dividing the mole fraction of *trans*-parinaric acid in the solid phase (0 22) by the preference of *trans*-parinaric acid for solid phase (about 3 1) yields the mole fraction 0 07 or 7% solid phase At 37 °C, approximately 7% and 3% of synaptic plasma membrane lipids appear to be in the solid phase (or clusters) and fluid phase, respectively Preincubation of synaptic plasma membrane with dolichol alters the lifetime, fractional fluorescence, and mole fraction of probe molecule for each component (Table IV) Dolichol does appear to increase the proportion of solid phase lipid slightly to 10% in the synaptic plasma membranes at 37 °C In addition, the fluidity of this solid phase may be increased (as indicated by decreased lifetime) These two effects on fluidity

TABLE IV

EFFECT OF DOLICHOL ON FLUORESCENCE LIFETIME OF *TRANS*-PARINARIC ACID IN SYNAPTIC PLASMA MEMBRANES AND LIPOSOMES

All conditions were as described in legend to Table III Values were obtained at 37 °C and represent the mean ± S E ($n = 3$) An asterisk refers to $P < 0.05$ by Student's t-test as compared to (−) dolichol

Membranes from	Dolichol	Lifetime (ns)		Fractional fluorescence		Mole fraction	
		τ_1	τ_2	F_1	F_2	X_1	X_2
Synaptic plasma	−	18 3 ± 0 4	2 0 ± 0 2	0 72 ± 0 02	0 22 ± 0 02	0 22 ± 0 02	0 78 ± 0 02
Synaptic plasma	+	14 7 ± 0 7 *	1 4 ± 0 7	0 81 ± 0 01 *	0 18 ± 0 01 *	0 30 ± 0 01 *	0 70 ± 0 01 *
Liposomes	−	16 9 ± 1 0	2 5 ± 0 8	0 72 ± 0 01	0 28 ± 0 01	0 28 ± 0 01	0 72 ± 0 01
Liposomes	+	25 7 ± 2 0 *	3 6 ± 0 4	0 57 ± 0 01 *	0 43 ± 0 01 *	0 16 ± 0 01 *	0 84 ± 0 01 *

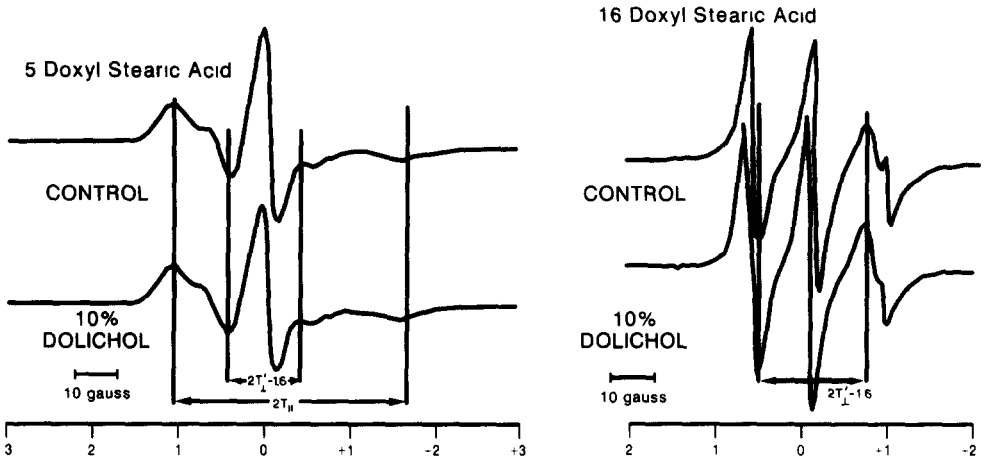

Fig 2 Effect of dolichol on ESR spectra of 5-doxylstearic acid Synaptic plasma membranes were incubated with buffer (control) or dolichol (1 10 ratio of dolichol to synaptic plasma membrane protein) as described in Mehtods Synaptic plasma membranes were then labeled with 5-doxylstearic acid and read at 37 °C Distances $2T'_{\perp} - 1\,6$ and $2T_{\parallel}$ were used to calculate the order parameter Each sample represents the average of four or five scans

Fig 3 Effect of dolichol on ESR spectra of 16-doxylstearic acid All procedures were as described in legend to Fig 2 except that 16-doxylstearic acid was used

are of an opposing nature and appear to cancel each other out, a possibility consistent with the data of Table III

The effect of dolichol on *trans*-parinaric acid lifetimes in liposomes derived from synaptic plasma membranes was not exactly the same as in the intact synaptic plasma membranes described above Dolichol increased the lifetime of and decreased the mole fraction of the long lifetime component Dolichol decreased the % solid phase from 9% to 5% while increasing the lifetime of the *trans*-parinaric acid in the solid phase Again, Table III indicates that these opposing effects on fluidity appeared to cancel each other

TABLE V

EFFECT OF DOLICHOL ON ORDER PARAMETER OF 5-DOXYLSTEARIC ACID IN SYNAPTIC PLASMA MEMBRANES OR LIPOSOMES

All procedures were as described in Methods

Membranes from	Probe	Dolichol	S
Synaptic plasma	5-NS	−	0 5966 ± 0 0016
Synaptic plasma	5-NS	+	0 5942 ± 0 0017
Liposomes	5-NS	−	0 5841
Liposomes	5-NS	+	0 5813

Electron spin resonance of doxylstearic acids in synaptosomal plasma membranes

Dolichol effects on *trans*-parinaric acid (static and dynamic properties) in synaptic plasma membrane may represent unique behavior of this fluorescent fatty acid probe Therefore, the effect of dolichols on doxyl-labeled fatty acid order was examined by ESR (Figs 2 and 3) The 5- and 16-doxylstearic acid spin labels report from different depths within the membrane bilayer [35] The 5-doxylstearic acid will position itself close to the membrane surface whereas the 16-doxylstearic acid reports on motion of the hydrophobic core of the

TABLE VI

EFFECT OF DOLICHOL ON $2T'_{\perp}$ OF 16-DOXYLSTEARIC ACID IN SYNAPTIC PLASMA MEMBRANES AND LIPOSOMES

All procedures were described in Methods $n = 3$ or 4 An asterisk refers to $P < 0\,05$ as compared to (−) dolichol

Membranes from	$2T'_{\perp}$		
	Dolichol		Difference
	−	+	
Synaptic plasma	22 84 ± 0 06	23 24 ± 0 03 *	0 40 ± 0 07
Liposomes	23 08 ± 0 07	23 65 ± 0 11 *	0 57 ± 0 08

membrane bilayer Dolichols did not have a significant effect in synaptic plasma membranes and liposomes using the 5-doxylstearic acid (Fig 2 and Table V) Dolichol significantly increased the $2T'_{\perp}$ of 16-doxylstearic acid in both synaptic plasma membranes and liposomes (Fig 3 and Table VI)

Discussion

The results presented here report for the first time the effects of dolichol on structural properties (static and dynamic) of a biological membrane, namely, the synaptosomal plasma membrane The effects can best be summarized as two-fold dolichols may either decrease (fluidize) or not alter the order of synaptic plasma membrane lipids, depending on the microenvironment of the reporter probe molecule used A number of points can be made from the data

First, dolichols are normally present in synaptic plasma membranes at very low quantities (391 ng/mg protein) Treatment of synaptic plasma membranes with buffer alone did not alter the content of dolichol while treatment with dolichol increased dolichol content

Second, dolichol significantly reduced the limiting anisotropy of diphenylhexatriene in synaptic plasma membranes by 0 012 at 37 °C (dolichol vs buffer) This result was confirmed with 16-doxylstearic acid which reports on motion deep in the bilayer hydrophobic core Although the above changes in diphenylhexatriene limiting anisotropy induced by dolichol appear small, this degree of change is approximately equivalent to either raising the temperature of the synaptic plasma membranes from 37 °C to 41 °C, or to adding an acute 100 mM dose of ethanol [36,37] In addition, the magnitude of this fluidization was larger than that noted for ethanol with ESR probes Ethanol (160 mM) decreased the order parameter of a doxyl fatty acid probe about 0 8% [38], whereas dolichols decreased the order parameter of diphenylhexatriene by 3 1% The effect of dolichol on synaptic plasma membranes was consistent with those reported for a number of model systems ESR probes [10], diphenylhexatriene polarization [39], differential scanning calorimetry [39,11], ^{31}P-NMR [11], freeze-fracture electron microscopy [11], and X-ray scattering [40] all indicated that dolichol (0–1 mole% of lipids) dramatically fluidized artificial phospholipid membranes This small quantity of dolichol promoted formation of nonbilayer hexagonal phase II in phosphatidylethanolamine and phosphatidylcholine/phosphatidylethanolamine mixtures [11,40] In this regard, both dolichols and temperature appear to affect the static (structural) but not dynamic aspects of diphenylhexatriene motion in model phospholipid membranes, as well as in a biological membrane

In contrast to the data obtained with diphenylhexatriene, dolichol did not alter either the limiting anisotropy or rotational rate (in radians/s) of *trans*-parinaric acid in synaptic plasma membranes This result was confirmed using 5-doxylstearic acid and ESR techniques The results obtained with fatty acid probes indicated that in the region close to the bilayer surface, dolichol did not change the rigidity and rate of motion of the microenvironment surrounding the fatty acids We and others [31–33], have shown earlier that *trans*-parinaric acid has a 3-fold preference for solid phase or clusters of lipids in membranes On the contrary, diphenylhexatriene partitions nearly equally well in all phases and thereby reports primarily on the predominantly fluid phase lipids in the synaptic plasma membranes It is somewhat surprising to find that at 37 °C, solid phase domains or clusters of lipids exist in synaptic plasma membrane because this membrane is highly enriched in unsaturated fatty acids This is especially so since a possible gel to liquid-crystalline phase transition in synaptic plasma membranes occurs near 24 °C, far below the 37 °C at which the dynamic measurements were taken However, clusters of solid lipids at 37 °C have been reported in retinal rod outer segment membranes which are also highly enriched in polyunsaturated fatty acids [34] Dolichol apparently affects lipids in fluid and solid phases differentially Such an observation for dissimilar behavior of diphenylhexatriene and *trans*-parinaric acid has been previously reported in fibroblast plasma membranes from aged vs young humans [41]

It should also be noted that a preliminary report from this laboratory indicated that dolichol had opposite effects on diphenylhexatriene and *trans*-parinaric acid limiting anisotropy, i e fluidizing vs rigidifying [42] Dolichol was reported

to increase the limiting anisotropy of *trans*-parinaric acid in synaptic plasma membranes This finding is not in agreement with the present report The discrepancy is due to a very large bovine serum albumin effect in the earlier report [42] In those experiments, the synaptic plasma membranes were incubated with dolichol or control buffer in the presence of bovine serum albumin as a vehicle The present results are internally consistent and in agreement with model membrane data [11] The data obtained with synaptic plasma membranes were also supported by mimicking dolichol effects on synaptic plasma membranes in liposomes prepared from the extracted lipids

The data reported here indicate that dolichols can have profound effects on the structure of a biological membrane As pointed out in the introduction, dolichol content of tissues is highly dependent upon age and disease status Dolichols appear to be localized in cellular membranes such as the plasma membranes One might predict that on the basis of the membrane structural alterations observed with dolichol, dolichols at low concentrations should also dramatically affect some membrane functions

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