PREFACE

Among the major scientific research efforts of the recent period has been the recognition of the importance of the "essential fatty acids" (EFA). The profound effects of these special chemical entities, and equally profound effects of their deficit, are appreciated by a variety of disciplines, including (but not necessarily limited to) lipid biochemistry, physiology, nutrition, psychology, psychiatry, and, perhaps most intensely, by the neurosciences at large. Functions of the central nervous system, in particular, may be seriously compromised by deficits in the levels of these FA or the ratio (or balance) among major constituents. The role of the polyunsaturated fatty acids (PUFA) α-linolenic acid (LNA; omega-3; n-3; 18:3n-3) and linoleic acid (LA; omega-6; n-6; 18:2n-6) and their metabolites has generated the most exciting findings. Health and medical implications related to these FA extend to visual development in infants, cognitive and emotional development, immunological responses, and cardiovascular health. Several foci of interest are worth noting at this point; foci that are represented in the chapters that follow and that mirror the directions in the field of FA research.

The experimental study of FA deficit has been characterized by investigations that utilize food deprivation or restrictions on nutritional intake, and by designs that have provided for dietary supplementation of the FA and/or their metabolites (especially DHA and its precursors EPA and LNA). Metabolic studies continue to address many of the unexplained complexities associated with the behavior performance observations in the laboratory. Among the questions of interest are: How do the EFAs get into the brain and other organs? What is the basis for the apparent selectivity of various organs, cells, and subcellular organelles for particular lipids and FA? Why is DHA (docosahexaenoic acid; 22:6n-3) concentrated in the brain? How can the adult brain maintain its DHA even when there is little support in the diet? How much can the metabolism of the precursors of DHA (e.g., LNA, EPA, etc.) support DHA composition in the brain in comparison to the incorporation of preformed DHA taken in the diet? In addition to their basic science value, these issues have practical implications for public health policy, such as the design of infant formulas.

The studies of supplementation have drawn attention to peripheral effects, such as the beneficial consequences of DHA in reducing cardiovascular mortality, reduction of immune and inflammatory responses, and influences in the management of diabetes. Supplementation effects also continue to be studied in order to better delineate complex behavioral patterns, with some critical insight on aggression, as but one example, in human studies.

Deprivation of n-3 in animal research has often been concentrated on the F2 offspring where demonstrable impairments in visual function and nonvisual cognitive behaviors have repeatedly been observed. Similar outcomes in human infants have been reported, with a pronounced increase in the frequency of randomized control trials being reported in the literature. Infant behavior appears to suffer quite seriously at the hands of nutritional deprivation, with some long-term followup studies suggesting that the early deficits appear to be maintained with functional loss in later years. The reader will soon discover that differences among outcome studies may be attributable, in part or in total, to variations in the test designs used to assess physiological or behavioral function. Often

the attempts to describe complex cognitive and emotional behaviors by use of learning and performance paradigms require a liberal interpretation of the results to support such assessments, which may be open to question or dispute.

Despite a number of known weaknesses, unexplained phenomena, and sorely needed pieces of information yet to be discovered, the present overview of activities in these areas allows one to justifiably conclude major advances in the chemistry and biochemistry of fatty acids have contributed to a considerable understanding about the metabolism and function of fatty acids and their impact on the physiology and behavior of whole organisms. The diversity of actions of fatty acids in many biological systems such as physiological, neurological, endocrinological, and immune begs for elucidation. The management of many chronic health issues will surely benefit from such knowledge in the near term. The purpose of *Fatty Acids: Physiological and Behavioral Functions* is to examine such a representative segment of the scientific aspects of this area, with topics ranging from molecular analyses to functional performance of physiological and cognitive behaviors. To assist the relative newcomer to the vocabulary of the field, we have provided a glossary at the end of the volume. Considerable additional helpful information is easily obtainable from many sources on the web, as even a brief search will indicate.

We hope that *Fatty Acids: Physiological and Behavioral Functions* will facilitate a consolidation of understanding among the separate disciplinary specialists, and will excite other investigators to enter this arena, so that even more dramatic advances and developments in chemistry, behavior, and health management will be forthcoming.

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2 Modulation of Receptor Signaling by Phospholipid Acyl Chain Composition

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1. INTRODUCTION

A guiding principle in the diverse investigations of biological molecules is that the functional and structural properties of macromolecular assemblies are determined by chemical and structural properties of the constituent molecules and the manner in which those molecules interact. In biological membranes, this requires an understanding of how membrane lipids, primarily phospholipids and cholesterol, and proteins interact with each other and among themselves, so as to carry out a wide range of biological functions. Most of the functions associated with biological membranes (e.g., signal transduction, ion movement, energy conversion, etc.) are carried out by membrane proteins. The phospholipids of neuronal and retinal cells are rich in highly unsaturated acyl chains, especially those of docosahexaenoic acid, 22:6n-3. A primary point of interest when considering receptor signaling is the role of highly unsaturated phospholipids and their effect on membrane protein function. Thus, the focus of this chapter will be on highly unsaturated acyl chains as components of phospholipids and their role in modulating membrane-associated signaling pathways. Therefore, the effects of polyunsaturated free fatty acids on the function of membrane proteins, such as L-type calcium channels (Kang & Leaf, 2000), γ-aminobutyric acid receptor (Nabekura et al., 1998), and voltage-gated potassium channels (Poling et al., 1996) will not be discussed.

Among the polyunsaturated fatty acid constituents of phospholipids, 22:6n-3 is among the most important. This fatty acid represents about 50% of the phospholipid acyl chain composition of the retinal rod outer segment (ROS) disk membrane (Stinson et al, 1991a) and about 40% of the acyl chains in synaptosomal membranes. It is also abundant in the membranes of neuronal and sperm cells (Salem, 1989). The importance of 22:6n-3 in retinal function is indicated by the difficulty in depleting this fatty acid in the retina by dietary manipulation (Stinson et al, 1991b; Bazan et al, 1993). The general importance of DHA in the nervous system is demonstrated by the observed visual and cognitive deficits in animals maintained on an n-3-deficient diet (for reviews, *see* Hamosh & Salem, 1998; Gibson & Makrides, 1998). Neuringer et al. (1984) have shown in nonhuman primates, that infants born to mothers raised on n-3-deficient diets have impaired visual response. Birch et al. (2000) have studied a group of preterm infants, which were fed formula, formula supplemented with 22:6n-3, or breast-fed. In cognitive and visual

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testing, the 22:6n-3 supplemented and breast-fed groups showed significantly better performance than the formula-fed group.

Several psychological disorders are currently being discussed in terms of the effect of the physical state of the membrane lipids on neurotransmitter receptor function. Hibbeln and Salem (1995) suggest that serotonin levels and membrane 22:6n-3 content are directly linked, whereby low 22:6n-3 yields low serotonin. This results in an individual being susceptible to depression or other affective diseases. These authors suggest that the depletion of 22:6n-3 induces a change in membrane physical properties, which, in turn, influences the function of either serotonergic receptors or serotonin reuptake systems.

In other literature, a conflict in the role of cholesterol in depression and suicide is evident. The rate of suicide and depression has been linked to total serum cholesterol levels. Suicide and violent behavior have been correlated with low serum cholesterol (Muldoon et al, 1990; Engelberg, 1992); however, a recent report indicates that the ratio of violent to nonviolent suicide rates correlates directly with total serum cholesterol (Tanskanen et al, 2000). The latter study suggests that the ratio of violent to nonviolent suicide rates, rather than the total suicide rate, might be a better correlative parameter with cholesterol levels.

The importance of investigating how cholesterol content and acyl chain composition alter the physical properties of membranes is highlighted by the functional deficits associated with 22:6n-3-deficient diets and the antisocial behavior associated with varied cholesterol levels. In the context of psychological disease and neurotransmitter receptor function, it is important to investigate how compositionally induced changes in membrane physical properties influence membrane-associated signaling processes.

The visual transduction pathway is the best characterized G-protein-coupled signal transduction system. Study of the visual receptor, rhodopsin, over the past several decades has made it the archetype of the growing superfamily of heptahelical G-protein-coupled receptors (reviewed in Litman & Mitchell, 1996a). The preeminent position of rhodopsin in this important superfamily will likely increase with the recent publication of the three-dimensional structure of rhodopsin (Palczewski et al., 2000). Many neurotransmitter receptors, as well as the olfactory and taste receptors, are members of this superfamily. Therefore, the effect of lipid membrane composition on various steps in visual signaling will be reviewed in some detail in this chapter. Given the similarity in mode of signaling, the observations made for the vision system should be of general applicability to other members of this receptor superfamily.

2. PROPERTIES OF POLYUNSATURATED PHOSPHOLIPID BILAYERS

In order to understand the biophysical mechanisms whereby polyunsaturated phospholipid acyl chains may alter membrane protein function, a number of investigators have examined model bilayer systems consisting of defined phospholipid composition. Measurements with a wide variety of techniques demonstrate that the introduction of a single *cis* double bond into a saturated acyl chain results in a large decrease in both acyl chain intramolecular order and the intermolecular acyl chain packing order in the liquid-crystalline or fluid phase. However, the effects of higher levels of unsaturation are not as widely agreed upon and appear to vary depending on the specific location of the double bonds and on whether one or both phospholipid acyl chains are unsaturated. Knowledge of the effects of acyl chain unsaturation on bilayer properties is especially incomplete for high levels of polyunsaturation (i.e., four or more double bonds). It does not appear that

the effects of a single double bond on acyl chain packing lead to a general description that can be used to explain the effects of high levels of polyunsaturation on molecular order and bilayer properties. However, direct investigation of the properties of highly polyunsaturated bilayers with a variety of techniques has significantly advanced our understanding of these systems in recent years.

Among the wide range of bilayer properties that are determined by the degree of acyl chain unsaturation, special emphasis in this chapter will be given to bilayer properties that have been proven or postulated to modulate the activity of one or more membrane proteins. Widespread attention has been accorded three physical mechanisms wherein a compositionally derived membrane property is correlated with membrane protein function: these are curvature strain (Epand, 1998; Gruner 1985), membrane thickness (Killian, 1998), and acyl chain packing (Hazel, 1995; Litman & Mitchell, 1996b). The effect on bilayer properties of the interaction of cholesterol with polyunsaturated acyl chains will also be reviewed.

2.1. General Physical Properties

Phospholipids containing unsaturated acyl chains melt or undergo the gel to liquid-crystalline phase transition, T_m, at much lower temperatures than phospholipids with saturated acyl chains containing an equal number of carbon atoms. For *sn-1* saturated, *sn-2* unsaturated phospholipids, the first double bond in the *sn-2* chain produces the greatest depression in the T_m. The T_m for 16:0,18:0 phosphatidylcholine (PC) is 321 K, whereas the T_m for 16:0,18:1n9 is 271 K, and the T_m for 16:0,18:2n6 PC is 255 K (Hernandez-Borrell et al., 1993). This trend is also observed for PCs with an 18:0 chain in the *sn-1* position (Niebylski & Salem, 1994). A simple, elegant molecular model has been presented recently that explains the T_m values of *sn-1* saturated, *sn-2* unsaturated phospholipids in terms of the number and position of *cis* carbon–carbon double bonds (Huang and Li, 1999). For diunsaturated PCs, the incremental reduction in T_m with increased number of double bonds is also quite small; the T_m for di18:2n6 PC is 216 K, whereas for di22:6n3 PC, the T_m is 205 K (Kariel et al., 1991; Keough et al., 1987). The relatively similar values of the T_m for dienes and higher polyenes show that the energetic difference between the gel and liquid crystalline states for these lipids are comparable. However, as detailed below, levels of phospholipid acyl chain unsaturation beyond two double bonds leads to alteration of other important bilayer properties.

High levels of phospholipid acyl chain unsaturation produce lipid bilayers with unique mechanical or material properties. Micropipet pressurization measurements demonstrate that bilayers composed of symmetrically substituted polyunsaturated acyl chain phospholipids are more flexible than bilayers composed of *sn-1*-saturated, *sn-2*-monounsaturated phospholipids of the same thickness (Rawicz et al., 2000). Similar measurements demonstrate that the area expansion modulus of di20:4 PC is much lower than that of bilayers composed of saturated or monounsaturated PCs, indicating that high levels of unsaturation yield less cohesive acyl chain packing, which results in a reduction in the energy required for membrane expansion (Needham & Nunn, 1990). The elastic area compressibility modulus is an indicator of the relative amount of energy required to compress a bilayer. The elastic area compressibility modulus for 18:0,22:6 PC is half that of 18:0,18:1 PC, and in 18:0,22:6 PC, the 18:0 chain is less compressible than the 22:6 chain (Koenig et al., 1997). These results demonstrate that the presence of polyunsaturated acyl chains reduces the amount of energy required to elastically deform a phospholipid bilayer and imparts a higher degree of compressibility.

It was recently shown that for bilayers composed of phospholipids with 18-carbon chains, increasing acyl chain unsaturation increases water permeability to such an extent that di18:3 PC is five times as permeable to water as 18:0,18:1 PC (Olbrich et al., 2000). Increased water permeation with increased acyl chain unsaturation does not appear to require both chains to be unsaturated, as 18:0,22:6 PC is about four times as permeable as 18:0,18:1 PC, but only about 30% less permeable than di22:6 PC (Huster et al., 1997).

2.2. Bilayer Thickness

Several investigators have reported variation in bilayer thickness with changing acyl chain unsaturation. X-ray diffraction measurements of peak-to-peak headgroup spacing show that a di18:2n6 PC bilayer is 2 Å thinner than a di18:1n9 PC bilayer. However, in a di20:4 PC bilayer, the bilayer thickness is reduced by only 0.5 Å, to 34.4 Å, relative to a di18:2n6 PC bilayer (Rawicz et al., 2000). This is consistent with deuterium nuclear magnetic resonance (NMR) measurements on *sn-1* saturated, *sn-2* unsaturated PCs, which indicate that 18:0,22:6 PC is only 1 Å thinner than 18:0,18:1 PC and that bilayer thickness is not further reduced after more than three double bonds are introduced to the *sn-2* acyl chain (Holte et al.,1995). Both x-ray diffraction and NMR measurements demonstrate that variation in acyl chain unsaturation produces relatively small changes in bilayer thickness.

2.3. Curvature Strain

Biological membranes are lamellar bilayers and lipid mixtures, extracted from biological membranes, form liquid-crystalline-phase bilayers under physiological conditions (McElhaney, 1984). However, some of the phospholipid components of biological membranes consist of nonbilayer phases in a purified form (Cullis & De Kruijff, 1979). Phospholipids with a headgroup cross-sectional area that is smaller than the cross-sectional area of the volume occupied by the acyl chains do not pack well into a planar bilayer; thus, they tend to undergo a lamellar to nonbilayer or inverted hexagonal (HII) phase transition. The stress that nonbilayer preferring lipids create in planar biological membranes has been termed curvature stress or curvature strain (Epand, 1998). A phosphatidylethanolamine (PE) headgroup is much smaller than a PC head group; thus, the presence of PE in a membrane contributes to curvature strain. A good measure of the relative curvature strain introduced by phospholipids is the temperature of their lamellar to hexagonal phase transition, T_H, with a lower T_H indicating greater curvature strain. For phospholipids with a PE headgroup, T_H is reduced as the unsaturation of the acyl chains increases (Dekker et al., 1983). This is consistent with the results of NMR measurements, which show that the cross-sectional area of the volume occupied by the phospholipid acyl chains increases with unsaturation (Holte et al., 1995; 1996). Curvature strain is also sensitive to the position of the unsaturation, as T_H for di18:1n9 PE is 20°C lower than for either di18:1n6 PE or di18:1n11 PE (Epand et al., 1996). Thus, membrane curvature strain may be altered by changes in either phospholipid headgroup composition or acyl chain unsaturation.

2.4. Acyl Chain Packing

Most biophysical studies of the effects of highly polyunsaturated phospholipid acyl chains on membranes have focused on changes in acyl chain packing, sometimes referred to as "fluidity," a term which has no validity at the dimensions of the bimolecular leaflets

forming a membrane. Changes in acyl chain packing are commonly characterized via changes in the steady-state anisotropy of a fluorescent probe like 1,3,5-diphenylhexatriene (DPH). A number of studies have demonstrated that a great deal more information about phospholipid acyl chain packing can be obtained from an analysis of the time-resolved decay of DPH fluorescence anisotropy in terms of an orientational distribution model (Mitchell & Litman, 1998a; Straume & Litman, 1987; Straume & Litman, 1988; van Ginkel et al., 1989). These studies show in detail that increased acyl chain polyunsaturation decreases the cohesion or tightness of acyl chain packing.

In *sn-1* saturated, *sn-2* unsaturated species, the orientation order decreases and the probe motional dynamics increase as the unsaturation of the *sn-2* chain is increased. The degree of orientation order drops dramatically in dipolyunsaturated species compared with PCs that contain a saturated *sn-1* chain and a polyunsaturated *sn-2* chain (Mitchell & Litman, 1998a). The reduction in acyl chain packing order upon going from a disaturated PC to a monounsaturated PC is well known, and symmetric, monounsaturated PCs such as di18:1n9 PC are often used in studies employing model membranes as representative of unsaturated phospholipid bilayers. However, the difference between a symmetrically substituted, highly polyunsaturated bilayer and a symmetrically substituted, monounsaturated bilayer is much greater than the difference between a monounsaturated bilayer and a bilayer where all of the acyl chains are saturated (Mitchell & Litman, 1998a).

The relative differences in acyl chain packing among highly unsaturated, monounsaturated, and saturated bilayers can be illustrated by comparing the angular orientations available to DPH (i.e., the DPH orientation distribution), for each bilayer. The relative probability of all DPH orientations, ranging from parallel to the bilayer normal (0°) to parallel to the plane of the membrane (90°) is derived from time-resolved measurements of DPH fluorescence. The 0° population is approximately parallel to the acyl chains, whereas the 90° population of DPH molecules is in the bilayer midplane (Mitchell & Litman, 1998a,b; Straume & Litman, 1987, 1988; van Ginkel et al., 1989). Comparisons of the DPH orientation probability for di14:0 PC with both di18:1 PC and di22:6 PC are shown in Fig. 1. The curves are the result of subtracting the orientation distribution for DPH in the di14:0 PC bilayer from the DPH orientation distribution in the unsaturated bilayer. Areas above the zero line indicate the range of angular orientations of DPH that are permitted to a greater extent in the unsaturated PCs than in di14:0 PC. Areas below the zero line indicate the range of angular orientations where DPH is has a higher probability of being located in di14:0 PC than it has being located in the unsaturated PCs. The curves in Fig. 1 show that the DPH population in the bilayer midplane, centered about 90°, is greater in both unsaturated bilayers than in di14:0 PC. However, the "difference curve" for di14:0 PC in Fig. 1 is the zero line, and the difference curve for di18:1 PC deviates much less from the zero line than the difference curve for di22:6 PC. This comparison shows that acyl chain packing in di18:1 PC is more similar to that found in di14:0 PC than it is to that found in di22:6 PC. In terms of important membrane properties, such as acyl chain packing, bilayer area per molecule, and water permeability, a bilayer consisting exclusively of monounsaturated acyl chains bears more resemblance to a saturated bilayer than it does to a bilayer containing highly polyunsaturated acyl chains, which is more representative of neuronal membranes.

In an early molecular modeling study, it was determined that the highest probability geometries for 22:6n-3 acyl chains were essentially linear, rigid configurations that would

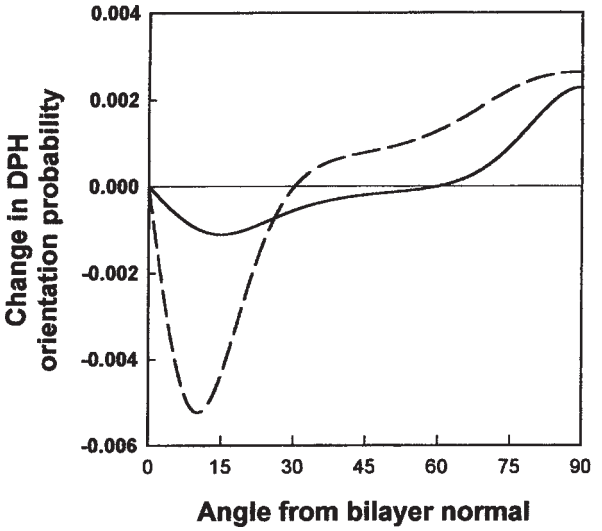

Fig. 1. Difference in orientation probability for the fluorescent membrane probe DPH in di14:0 PC compared to di18:1 PC (—) and di14:0 PC compared to di22:6 PC (---). Each curve is the result of subtracting the probability distribution for DPH orientation in the di14:0 PC from the probability distribution for DPH orientation in the unsaturated bilayer. Regions above the zero line correspond to a range of angular orientations that have a higher probability in the unsaturated bilayer than in di14:0 PC.

facilitate the formation of tightly packed two-dimensional arrays (Applegate & Glomset, 1986). However, the experimental evidence published to date, regarding either 22:6n-3 chain packing or conformation indicates that 22:6n-3 acyl chains have a high degree of flexibility and do not have a high probability of being in an extended linear conformation. A series of NMR measurements have shown that 22:6n-3 acyl chains are loosely packed in fluid-phase bilayers and individual 22:6n-3 acyl chains have very low orientational order (Holte et al., 1995; Holte et al., 1996; Huster et al., 1998). The area of the bilayer occupied by a phospholipid molecule is a strong indicator of the orderliness of acyl chain packing. The area per molecule for 18:0,22:6 PC is approx 12% higher than in 18:0,18:1 PC (Koenig et al., 1997), indicating a higher degree of disorder in the 18:0,22:6 PC. Monolayer studies show that, at the lateral surface pressure corresponding to biological membranes, the area per molecule for di22:6 PC is 50% greater than for 18:0,22:6 PC (Zerouga et al., 1995), which is consistent with greater bilayer free volume in a di22:6 PC bilayer than in a 16:0,22:6 PC bilayer, particularly in the bilayer midplane (Mitchell & Litman, 1998a).

Both increased temperature and increased acyl chain unsaturation introduce disorder in phospholipid acyl chain packing; thus, their effects on bilayer properties are often compared and discussed as being equivalent perturbations on the bilayer structure. However, detailed studies of the effect of these two factors on both intrachain order and acyl chain packing reveal significant differences. The difference between temperature-induced disorder and *sn-2* unsaturation-induced disorder of the saturated *sn-1* acyl chain is illustrated in Fig. 2A. The difference in NMR order-parameter data is plotted for each methyl group of the saturated 18:0 *sn-1* acyl chain. The two difference curves show that the disorder caused by exchanging 18:1n-9 at the *sn-2* position for 22:6n-3, occurs mainly

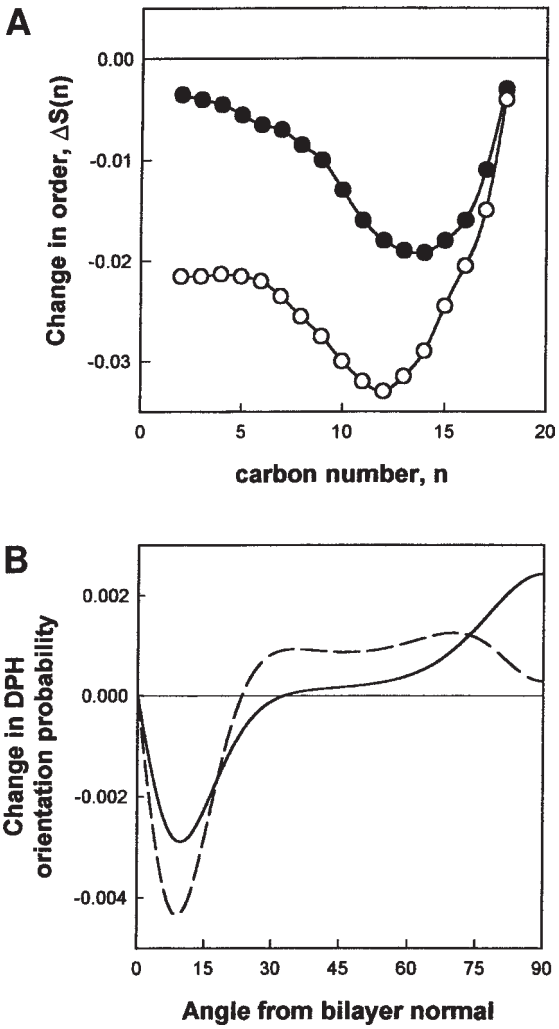

Fig. 2. Increased acyl chain unsaturation and increased temperature produce dissimilar increases in acyl chain disorder. **(A)** Difference order-parameter profiles, $\Delta S(n)$, of deuterium NMR measurements on perdeuterated 18:0 in the *sn-1* position as a function of changes in temperature and unsaturation at the *sn-2* position. ●: $\Delta S(n)$ between $18{:}0_{d35}$, 22:6 PC and $18{:}0_{d35}$, 18:1 PC; ❍:$\Delta S(n)$ for $18{:}0_{d35}$,18:1 PC between 27°C and 47°C. Carbon atoms are numbered beginning at the glycerol backbone. (Data from Gawrisch and Holte, 1996; used by permission of K. Gawrisch). **(B)** Difference in orientation probability for the fluorescent membrane probe DPH. Orientation distribution of DPH in 16:0,18:1 PC at 40°C minus that in 20°C (—), and the distribution of di22:6 PC minus that of 16:0,18:1 PC at 20°C (---).

in the terminal half of the acyl chain, whereas the disorder induced by raising the temperature occurs over the entire chain.

Elevated temperature and increased unsaturation also have distinct effects on the average acyl chain packing. DPH orientation difference curves that compare the DPH orientation probability in 16:0,18:1 PC between 20°C and 40°C, and between 16:0,18:1 PC and di22:6 PC are shown in Fig. 2B. The solid curve shows that raising the temperature

reduces the population oriented parallel to the acyl chains and increases the population in the bilayer midplane, oriented parallel to the bilayer surface. This shift indicates that an increase in temperature alters acyl chain packing in such a way that the bilayer midplane can accommodate a larger fraction of the DPH molecules. However, the distributions remain fairly narrow. The region below the zero line in the dashed curve shows that the presence of 22:6n-3 acyl chains also reduces the DPH population oriented parallel to the acyl chains. However, the dashed curve above the zero line shows that the presence of 22:6n-3 acyl chains produces a pronounced broadening of both orientational modes, rather than just a shift of DPH from the parallel mode to the perpendicular mode. The two comparisons in Fig. 2 demonstrate that elevated temperature and increased acyl chain unsaturation have distinct effects on both acyl chain disorder and average acyl chain packing and that the properties of highly unsaturated membranes are not equivalent to more saturated membranes at a higher temperature.

2.5. Interaction With Cholesterol

It is well established that cholesterol has a strong ordering effect on saturated phospholipid acyl chains in the fluid phase. This results in condensation of phospholipid monolayers and a reduction in enthalpy of the main gel to liquid-crystalline phase transition. These effects are reduced as *sn-2* acyl chain unsaturation is increased for phospholipids with a saturated *sn-1* chain (Hernandez-Borrell et al., 1993; Smaby et al., 1997), although the proximity of the double bonds to the headgroup is as important as the number of double bonds (Stillwell et al., 1994).

For all symmetrically substituted, unsaturated PCs, the chain-ordering effect of cholesterol is greatly reduced when compared with the corresponding *sn-1* saturated, *sn-2* unsaturated PC, and the effect of cholesterol decreases as the level of unsaturation increases (Mitchell & Litman, 1998b). A few studies have examined the effects of cholesterol in bilayers consisting of dipolyunsaturated PCs. All of these studies demonstrate that even at concentrations above 30 mol%, cholesterol has very little effect on the acyl-chain-packing properties of dipolyunsaturated bilayers. In both di20:4 PC and di22:6 PC, cholesterol has almost no effect on the gel–liquid-crystalline phase transition (Kariel et al., 1991), causes minimal change in acyl chain packing (Mitchell & Litman, 1998b), and causes only a small increase in the monolayer elastic area compressibility modulus (Smaby et al., 1997). In 18:0,18:1 PC bilayers, 50 mol% cholesterol increases the elastic area expansion modulus by 600 dyn/cm, whereas in di20:4 PC, 50 mol% cholesterol increases this parameter by only 50 dyn/cm (Needham & Nunn, 1990). The best explanation of these observation comes from a deuterium NMR study, which showed that cholesterol is soluble in di20:4n6 PC only to 15 mol% and that the molecular organization of cholesterol in this bilayer is profoundly different from that observed in *sn-1* saturated, *sn-2* polyunsaturated bilayers (Brzustowicz et al., 1999).

In recent years, much evidence has accumulated for lateral membrane domains that differ in their relative cholesterol content (Schroeder et al., 1995). In addition, it has been proposed that high levels of *sn-2* unsaturation may promote formation of microdomains, in which the saturated *sn-1* chains preferentially interact with each other (Litman et al., 1991). Several studies of cholesterol in bilayers containing high levels of polyunsaturation have reported evidence of lateral domains, which are driven by the preference of cholesterol for saturated acyl chains over polyunsaturated acyl chains (Huster et al., 1998; Mitchell & Litman, 1998b; Polozova & Litman, 2000; Zerouga et al., 1995). The recent

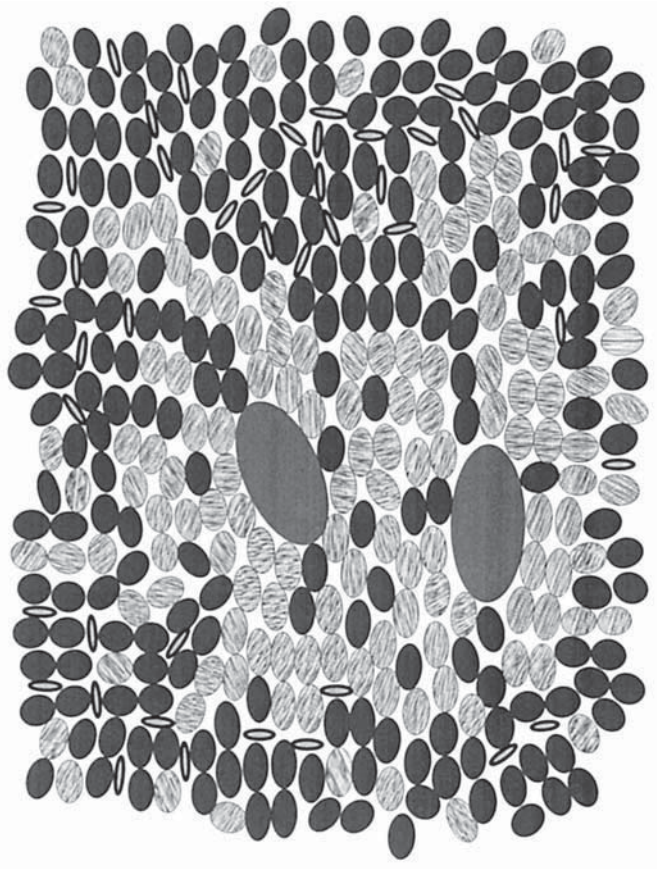

Fig. 3. Schematic representation of lateral domains in a bilayer consisting of di16:0 PC (dark ovals), di22:6 PC(striated ovals), cholesterol (small, light ovals) in a 7 : 3 : 3 ratio and rhodopsin (large gray ovals) at a 100 : 1 ratio of PC : rhodopsin. Rhodopsin is in a cluster, highly enriched in 22:6n-3 acyl chains, whereas cholesterol is mainly associated with the saturated 16:0 acyl chains. The enrichment of di22:6 PC in the cluster around rhodopsin is enhanced about six times relative to the bulk concentration. The cluster extends about three layers around rhodopsin.

work of Polozova and Litman (2000) is especially significant in terms of biological mechanisms, because it was found that in a mixed PC system composed of di16:0 PC and di22:6 PC, lateral domain behavior was observed only when both cholesterol and the integral membrane protein rhodopsin were included in the bilayer. A conceptual diagram of the proposed protein-containing microdomains is shown in Fig. 3. The observation that rhodopsin was essential for the formation of domains and showed a distinct preference for di22:6 PC indicates a mechanism whereby changes in either phospholipid acyl chain unsaturation or membrane cholesterol could control membrane domain formation and, thereby, integral membrane protein function.

3. EFFECTS OF ACYL CHAIN COMPOSITION ON MEMBRANE PROTEIN FUNCTION

Numerous studies have been published describing modulation of membrane protein function by changes in the degree of unsaturation of the phospholipid acyl chains. It is

convenient to divide these studies into two types, those that investigated membrane protein function in natural membranes and those that examined the function of purified membrane proteins reconstituted with defined phospholipids. The literature abounds with examinations of the effects of diets or drugs on a wide range of physiological and behavioral outcomes. In this chapter, we are concerned with the relatively few studies that have examined the isolated function of one or more specific receptors and performed an analysis of the composition of the receptor's host membrane.

Several different receptor systems in the heart have been examined in this kind of detail. The amount of 22:6n-3-containing phospholipid in the sarcolemmal membranes of rats was elevated by injections of hydrocortisone, and this was accompanied by a downregulation of β-adrenergic receptors (Skuladottir et al., 1993). This finding is supported by the results of a detailed examination of 22:6n3-supplemented cardiomyoctes (Grynberg et al., 1995). Fatty acid enrichment produced cells in which 20% of the total fatty acids were 22:6n3. The α-adrenergic system was unaffected, but the β-adrenergic receptors had a decreased affinity for ligand. In a study of streptozotocin-induced diabetes mellitus, the sarcoplasic reticulum membranes of treated rats showed a loss of arachidonic acid, 20:4n6, acyl chains and an increase in 22:6n-3 (Kuwahara et al., 1997). This was accompanied by decreased sodium/potassium-ATPase activity and a reduction in calcium uptake.

A number of different types of membrane protein have been examined in reconstituted lipid vesicles containing high levels of acyl chain unsaturation, 20:4n6 or 22:6n3. These include sarcoplasmic reticulum calcium ATPase (Matthews et al., 1993), protein kinase C (PKC) (Giorgione et al., 1995; Slater et al., 1994;), gramicidin (Cox et al., 1992), and rhodopsin (Brown, 1994; Gibson & Brown, 1993; Litman & Mitchell, 1996b; Mitchell et al., 1990; Mitchell et al., 1992; O'Brien et al., 1977; Wiedmann et al., 1988). Higher levels of acyl chain unsaturation promote membrane protein function in all of these membrane proteins except calcium ATPase. Calcium ATPase was reconstituted into PCs with a 16:0 acyl chain at the *sn-1* position and 18:0, 18:1n9, 18:2n6, 20:4n6, or 22:6n3 acyl chain at the *sn-2* position. Enzyme function obtained with 18:1n9 or 18:2n6 at the *sn-2* position was more than 10 times higher than that obtained with 20:4n6 or 22:6n3 at the *sn-2* position (Matthews et al., 1993). In the other studies cited above, the highest level of protein function was obtained in the most highly unsaturated bilayer examined. However, there is no agreement among these studies regarding the physical property of the bilayer and the associated forces, which promote membrane protein function in highly unsaturated bilayers. The following subsections describe studies, wherein the authors have attributed their observations relative to membrane protein function to bilayer thickness, curvature stress, or acyl chain packing properties (e.g., acyl chain packing free volume).

3.1. Bilayer Thickness

Relatively large changes in membrane thickness have been demonstrated to alter the function of integral membrane proteins. An example of the magnitude of the change in membrane thickness needed to alter protein function is provided by studies of the sarcoplasmic reticulum calcium ATPase. Activity of this integral membrane protein in bilayers with symmetrically substituted, monounsaturated acyl chains with 16, 18, or 20 carbons is nearly constant. However, when the acyl chains are shortened to 14 carbons or lengthened to 22 carbons, activity is reduced by more than a factor of 3 (Lee, 1998).

This indicates that extreme changes in membrane thickness can alter protein function. However, the small changes in membrane thickness caused by physiologically relevant changes in acyl chain unsaturation are well within the range of constant calcium ATPase activity and seem unlikely to alter the function of other integral membrane proteins.

3.2. Curvature Strain

The activity of several different membrane proteins has been correlated with the propensity of the lipids composing the membrane to form nonlamellar structures or the curvature strain of the host bilayer (Epand, 1998; Li et al., 1995). Examples of alteration of membrane protein function via variable acyl chain composition, which were interpreted as changes in curvature stress, are provided by mitochondrial ubiquinol–cytochrome-*c* reductase and H^+ ATPase. When these protein are reconstituted into liposomes of di18:1n9-*cis* PC and di18:1n9-*trans* PE, their activity is increased as the percentage of di18:1n9-*cis* PE is raised, whereas the addition of di18:1n9-*trans* PE had no effect (Li et al., 1995). This demonstrated that the effect was not the result of the addition PE headgroups to the bilayer and suggested that it was related to the greater propensity of di18:1n9-*cis* to form a nonbilayer phase.

Epand and co-workers found that 22:6n-3 acyl chains produced the highest level of PKC function when incorporated into PE, but not PC, and the activity was correlated with increased partitioning of PKC to the membrane (Giorgione et al., 1995). Stubbs and co-workers found that PKC activity was optimal when the membrane had a combination of headgroup spacing and bilayer curvature, which could be obtained with a mixture of PEs and PCs containing a 22:6n3 acyl chain (Slater et al., 1994). The rate at which gramicidin converted from a nonchannel to a channel-forming conformation was highest in PC membranes, which contained 22:6n3 acyl chains or PE phospholipids, and it was proposed that this is the result of increased curvature stress (Cox et al., 1992).

A recent detailed study of calcium-ATPase function and phospholipid motion concluded that PE headgroups promote the activity of calcium ATPase by specific noncovalent interactions, rather than by bilayer curvature stress, as had been previously proposed (Hunter et al., 1999). In biological membranes, which contain both PE and PC and a range of acyl chain compositions, the effects of polyunsaturated acyl chains and PE headgroups on curvature stress can be difficult to assess, because of the complex influence the acyl chains of one phospholipid species exert on those of other species (Holte et al., 1996; Separovic & Gawrisch, 1996). Although quantitative correlation of protein function with curvature stress derived from acyl chain unsaturation remains difficult, there is ample evidence to suggest that the curvature stress induced by high polyunsaturated acyl chains could be functionally significant. The challenge is to measure both alterations in function and curvature stress in the same system, so as to allow a direct correlation between these membrane properties.

Rhodopsin, the light receptor in the G-protein-coupled visual transduction system, has been studied extensively in reconstituted systems. Light converts rhodopsin's antagonist, 11-*cis* retinal, to the agonist, all-*trans* retinal, resulting in the formation of activated receptor. The conformation of activated receptor, which binds the visual G protein, metarhodopsin II (MII), exists in equilibrium with an inactive conformation, metarhodopsin I (MI). Thus, the extent of functional activation is given by the equilibrium between MI and MII. The general observation is that the formation of MII is highest in bilayers composed of phospholipids with 22:6n3 acyl chains. In a series of experiments,

Brown and co-workers demonstrated that components that produce curvature strain could replace 22:6n3 acyl chains without compromising the extent of formation of MII. The ability of PE headgroups to support optimal rhodopsin function was analyzed in mixtures of di18:1 PC and di18:1 PE. MII formation in bilayers composed of 75% di18:1 PE and 25% di18:1 PC was found to be equivalent to that observed in bilayers where 50% of the acyl chains are 22:6n-3 (Brown, 1994). In a second set of measurements, it was found that diphytanoyl PC was as effective as di22:6 PC in promoting MII formation in PC/PE/PS mixtures, which mimicked the headgroup composition of the native rod outer segment disk membrane (Brown, 1994). Based on these findings, Brown has proposed that MII formation is facilitated by a lipid bilayer, which has curvature strain because the formation of MII results in a release of the curvature strain in the bilayer adjacent to rhodopsin (Brown, 1994). Although these studies provide a strong inference relative to the role of curvature strain in modulating MII formation, this interpretation suffers from a lack of direct measurements of curvature strain on the reconstituted membrane systems used in this study.

3.3. Acyl Chain Packing

An alternative explanation of the promotion of MII formation comes from direct measurements of acyl chain packing in rhodopsin-containing bilayers composed of a series PCs with varying levels of acyl chain unsaturation and cholesterol (Litman & Mitchell, 1996b; Mitchell et al., 1990; Mitchell et al., 1992). Acyl chain packing was characterized by analyzing the decay of fluorescence anisotropy of the membrane probe DPH in terms of the orientation distribution of DPH in the bilayer. The orientation distribution of DPH was summarized by a parameter, F_v, which is a measure of the difference between the DPH orientation distribution in the sterically restricted space of the bilayer and that which would be observed for an unrestricted free-tumbling DPH molecule (Mitchell & Litman, 1998a; Straume & Litman, 1987). F_v is positively correlated with the acyl chain packing free volume. Both MII formation and, F_v were measured in bilayers composed of a variety of PCs with and without cholesterol.

Examples of the effects of acyl chain composition and cholesterol on the MI–MII equilibrium constant, K_{eq}, are summarized in Fig. 4. The equilibrium constant for the formation of MII from its inactive precursor MI, K_{eq}, was measured in these rhodopsin-containing vesicles. For each acyl chain composition, K_{eq} was determined to be linearly correlated with F_v in a manner that was independent of cholesterol content (Mitchell et al., 1990; Mitchell et al., 1992). These correlations demonstrate that each acyl chain composition produces a unique correlation between MII formation and acyl chain packing that is not altered by cholesterol. The bars in Fig. 4 demonstrate that high levels of acyl chain unsaturation enhance MII formation. The linear correlations between K_{eq} and F_v demonstrate that this enhancement is related to the higher degree of disorder in acyl chain packing, resulting in increased acyl chain packing free volume. An unexplored question is whether the enhancement of MII formation by PE phospholipid groups is also correlated with acyl chain packing disorder.

In visual signal transduction, activation proceeds from the receptor, rhodopsin, to the effector, phosphodiesterase (PDE), via the visual G protein, G_t. Each MII sequentially binds and activates up to 100 G_t, thus MII–G_t binding initiates the first stage of signal amplification in the visual pathway. Litman et al. (2001) have studied the phospholipid acyl chain dependence of the kinetics of formation of both the MII conformation and the

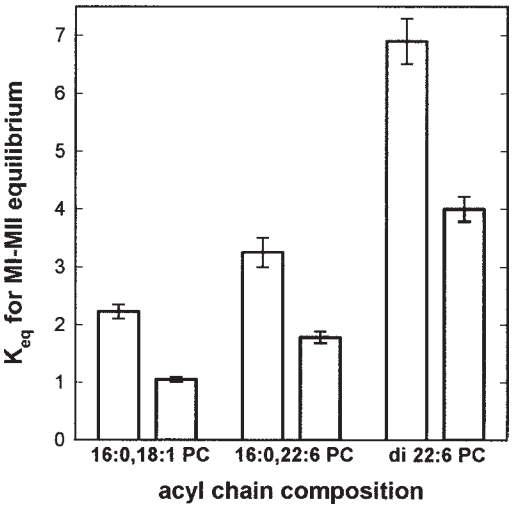

Fig. 4. Examples of the effects of acyl chain composition (white bars) and cholesterol (gray bars) on K_{eq} for the MI–MII equilibrium of photolyzed rhodopsin at 37°C. Higher values of K_{eq} correspond to higher equilibrium concentrations of MII, the state of photolyzed rhodopsin that participates in visual signal transduction by binding the visual G protein.

MII–G_t complex. The temporal nature of the interaction of MII and G_t is characterized by the ratio of the rate of formation of the MII–G_t complex divided by the formation rate of MII. This ratio varied from 1.39 to 4.95 in native disk membranes and 18:0,18:1 PC bilayers, respectively. In 18:0,22:6 PC bilayers, the ratio had an intermediate value of 3.46.

An important feature of 22:6n-3-containing bilayers is the ability to buffer the inhibitory effects of cholesterol. The inclusion of 30 mol% cholesterol in an 18:0,22:6 PC bilayer had relatively little effect on MII coupling to G_t, whereas this level of cholesterol in 18:0,18:1 PC bilayers resulted in a ratio of 9.1 and an increase in lag time for complex formation of 6.5-fold, relative to native disk membranes. Complex formation involves MII and G_t diffusion in the surface of the membrane. The increased lag time suggests that the diffusion process is dramatically slowed by the presence of cholesterol in 18:0,18:1 PC, whereas this process is relatively unaffected by cholesterol in 18:0,22:6 PC bilayers. A delay in the coupling of MII with G_t decreases the response time of the pathway. In addition to the kinetics of MII–G_t complex formation, a reduced binding affinity of MII to G_t was observed in 18:0,18:1 PC relative to 18:0,22:6 PC bilayers (Niu, Mitchell, and Litman, unpublished results). The addition of cholesterol reduced the binding affinity of MII for G_t to a greater degree in 18:0,18:1 PC bilayer than in 18:0,22:6 PC bilayers. Thus, signal amplification along the pathway will be reduced in less unsaturated bilayers. These data demonstrate explicitly that 22:6n-3-containing phospholipids can buffer the inhibitory effects of cholesterol in a signaling pathway and highlight the potential importance of 22:6n-3 acyl chains in optimizing both the response time and magnitude of response in signaling pathways.

In the visual pathway, the activity of the PDE is a measure of the integrated pathway activity. Litman et al. (2001) studied the phospholipid acyl chain dependence of the light-stimulated PDE activity. This study was carried out in reconstituted systems, which

included G_t, PDE, and rhodopsin in unilamellar vesicles, whose phospholipid composition was either 16:0,18:1 PC or 16:0,22:6 PC. Each rhodopsin absorbing a photon is analogous to an agonist-bound receptor. A level of 1 in 1000 rhodopsin molecules activated by light produced 59% of the activity obtained in native disk membranes for rhodopsin in 16:0,22:6 PC bilayers and only 26% of disk activity for rhodopsin in 16:0,18:1 PC bilayers. Under conditions of saturating stimulation, 97% of the activity of native disk-membrane response was observed in the 16:0,22:6 PC vesicles system, whereas only 50% of the disk activity was seen in 16:0,18:1 PC vesicles. Here again, the system properties are optimized in 22:6n-3-containing bilayers.

The presence of lateral domains in di22:6 PC bilayers demonstrates an additional mechanism whereby 22:6n-3-containing bilayers can enhance signaling processes. If, in addition to rhodopsin, G_t and PDE also show preferential partitioning into regions rich in 22:6n-3, then lateral domain formation will increase the efficiency of association of these proteins by reducing the diffusion pathway for their interaction and increasing their effective concentration in the region of the microdomains.

4. SUMMARY

Highly polyunsaturated phospholipids produce membranes with several unique characteristics, and these characteristics are beneficial to many biological membrane functions. The high levels of 22:6n-3 acyl chains in the membrane phospholipids of the nervous system and retina suggest that these phospholipids play an important structural role. There is general agreement that the presence of polyunsaturated acyl chains in the phospholipids of membranes imparts a variety of unique features to these membranes. The specialized physical properties of highly polyunsaturated bilayers include increased bilayer area per headgroup, increased water permeability, higher degree of acyl chain flexibility, dynamics and disorder, sharply reduced interaction with cholesterol, and a tendency to enhance the formation of lateral domains. Although the actual forces that modulate protein function are still under investigation, it is clear that the unique properties imparted to biological membranes by the presence of polyunsaturated acyl chains and, in particular, 22:6n-3 chains play a fundamental role in determining membrane protein function.

The visual pathway, which is a prototypical G-protein-coupled receptor system, is one of the best characterized of this family of receptor systems. Various steps in this pathway are optimized in 22:6n-3-containing bilayers. The highest levels of MII formation in reconstituted systems are observed in 22:6n-3-containing bilayers (Litman & Mitchell, 1996b). The 22:6n-3-containing bilayers exhibited increased levels of MII–G_t complex formation and more favorable kinetic coupling of MII and G_t, relative to less unsaturated membrane systems (Litman et al., 2001). MII and G_t must interact rapidly to form a complex upon formation of MII in order to make signaling along the pathway efficient. As the bilayer acyl chains become less unsaturated, this process becomes delayed, introducing a lag time in the signaling process. Reduced affinity of the receptor, MII, for G_t will result in less amplification in the pathway, resulting from there being fewer G_ts activated. Observations made in reconstituted systems are in good agreement with electroretinogram (ERG) measurements made on n-3-deficient animals, where reduced signal amplitude and a lag time in signal development are seen. In view of the similarities between the visual system and other G-protein-coupled receptor pathways, the findings

in the vision pathway ought to be applicable to neurotransmitter receptors in this superfamily. This extrapolation is supported by studies evaluating olfactory discrimination of rats raised on either an n-3-deficient or n-3-adequate diet. The n-3-adequate group made fewer errors in odor discrimination tests than the n-3-deficient group (Greiner et al, 1999). The olfactory bulb of rats raised on an n-3-deficient diet showed an 82% loss of 22:6n-3 relative to rats raised on an n-3-adequate diet. Olfactory and visual signaling are both G-protein-coupled receptor pathways and both are less sensitive in 22:6n-3-deficient animals.

The finding that 22:6n-3-containing bilayers buffer the inhibitory effects of cholesterol has strong implications for psychological disorders that are associated with variable levels of cholesterol. Under conditions of reduced levels of 22:6n-3 in n-3-deficiency and an increased cholesterol level, a reduced sensitivity in G-protein-coupled receptor signaling would be anticipated. Thus, the effectiveness of serotonin or other associated neurotransmitters would be decreased, potentially inducing some of the observed psychological dysfunction associated with cholesterol.

The studies described herein provide insight into the mechanism whereby 22:6n-3-containing phospholipids optimize membrane-associated signaling processes. Studies of both odor discrimination in n-3-deficient rats (Greiner et al, 1999) and visual deficits in n-3-deficient rhesus monkeys (Neuringer et al., 1984) and formula-fed infants (Birch et al., 2000) demonstrate a marked desensitization of two distinct G-protein-coupled signaling pathways to 22:6n-3 deficiency. It is anticipated that the sensitivity of G-protein-coupled receptor systems to levels of 22:6n-3 in membrane phospholipids will be of a general nature and this phenomenon may provide an explanation of the deficiencies in cognitive processes observed in n-3 deficiency.

REFERENCES

Applegate KR, Glomset JA. Computer-based modeling of the conformation and packing properties of docosahexaenoic acid. J Lipid Res 1986; 27:658–680.

Bazan NG, de Turco R, Gordon WC. Pathways for the uptake and conservation of docosahexaenoic acid in photoreceptors and synapses: biochemical and autoradiographic studies. Can J Physiol Pharmacol 1993; 71:690–698.

Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental Development in infants. Dev Med Child Neurol 2000; 42:174–181.

Brown MF. Modulation of rhodopsin function by properties of the membrane bilayer. Chem Phys Lipids 1994; 73:159–180.

Brzustowicz MR, Stillwell W, Wassall SR. Molecular organization of cholesterol in polyunsaturated phospholipid membranes: a solid state 2H NMR investigation. FEBS Lett 1999; 451:197–202.

Cox KJ, Ho C, Lombardi V, Stubbs CD. Gramicidin conformational studies with mixed-chain unsaturated phospholipid bilayer systems. Biochemistry 1992; 31:1112–1117.

Cullis PR, de Kruijff B. Lipid polymorphism and the functional roles of lipids in biological membranes. Biochim Biophys Acta 1979; 559:399–420.

Engelberg H. Low serum cholesterol and suicide. Lancet 1992; 339:727–729.

Dekker CJ, Geurts van Kessel WS, Klomp JP, Pieters J, De Kruijff B. Synthesis and polymorphic phase behaviour of polyunsaturated phosphatidylcholines and phosphatidylethanolamines. Chem Phys Lipids 1983; 33:93–106.

Epand RM, Fuller N, Rand RP. Role of the position of unsaturation on the phase behavior and intrinsic curvature of phosphatidylethanolamines. Biophys J 1996; 71:1806–1810.

Epand RM. Lipid polymorphism and protein-lipid interactions. Biochim Biophys Acta 1998; 1376:353–368.

Gawrisch K, Holte LL. NMR investigations of non-lamellar phase promoters in the lamellar phase state. Chem Phys Lipids 1996; 81:105–116.

Gibson R, Makrides M. The role of long chain polyunsaturated fatty acids (LCPUFA) in neonatal nutrition. Acta Paediatr 1998; 87:1017–1022.

Gibson NJ, Brown MF. Lipid headgroup and acyl chain composition modulate the MI-MII equilibrium of rhodopsin in recombinant membranes. Biochemistry 1993; 32:2438–2454.

Giorgione J, Epand RM, Buda C, Farkas Y. Role of phospholipids containing docosahexaenoyl chains in modulating the activity of protein kinase C. Proc Natl Acad Sci USA 1995; 92:9767–9770.

Gruner SM. Intrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids. Proc Natl Acad Sci USA 1985; 82:3665–3669.

Greiner RS, Moriguchi T, Hutton A, Slotnick BM, Salem N Jr. Rats with low levels of brain docosahexaenoic acid show impaired performance in olfactory-based and spatial learning tasks. Lipids 1999; 34:S239–243.

Grynberg A, Fournier A, Sergiel JP, Athias P. Effect of docosahexaenoic acid and eicosapentaenoic acid in the phospholipids of rat heart muscle cells on adrenoceptor responsiveness and mechanism. J Mol Cell Cardiol 1995; 27:2507–2520.

Hamosh M, Salem N Jr. Long-chain polyunsaturated fatty acids. Biol Neonate 1998; 74:106–120.

Hazel JR. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Ann Rev Phys 1995; 57:19–42.

Hernandez-Borrell J, Keough KM. Heteroacid phosphatidylcholines with different amounts of unsaturation respond differently to cholesterol. Biochim Biophys Acta 1993; 1153:277–282.

Hibbeln JR, Salem N Jr. Dietary polyunsaturated fatty acids and depression: When cholesterol does not satisfy. Am J Clin Nutr 1995; 62:1–9.

Holte LL, Peter SA, Sinnwell TM, Gawrisch K. ^{2}H nuclear magnetic resonance order parameter profiles suggest a change of molecular shape for phosphatidylcholines containing a polyunsaturated acyl chain. Biophys J 1995; 68:2396–2403.

Holte LL, Separovic F, Gawrisch K. Nuclear magnetic resonance investigation of hydrocarbon chain packing in bilayers of polyunsaturated phospholipids. Lipids 1996; 31:S199–S203.

Huang C, Li S. Calorimetric and molecular mechanics studies of the thermotropic phase behavior of membrane phospholipids. Biochim Biophys Acta 1999; 1422:273–307.

Hunter GW, Negash S, Squier TC. Phosphatidylethanolamine modulates Ca-ATPase function and dynamics. Biochemistry 1999; 38:1356–1364.

Huster D, Jin AJ, Arnold K, Gawrisch K. Water permeability of polyunsaturated lipid membranes measured by ^{17}O NMR. Biophys J 1997; 73:855–864.

Huster D, Arnold K, Gawrisch K. Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipid mixtures. Biochemistry 1998; 37:17,299–17,308.

Kang JX, Leaf A. Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids. Am J Clin Nutr 2000; 71:202s–207s.

Kariel N, Davidson E, Keough KM. Cholesterol does not remove the gel–liquid crystalline phase transition of phosphatidylcholines containing two polyenoic acyl chains. Biochim Biophys Acta 1991; 1062:70–76.

Keough KM, Kariel N. Differential scanning calorimetric studies of aqueous dispersions of phosphatidylcholines containing two polyenoic chains. Biochim Biophys Acta 1987; 902:11–18.

Killian JA. Hydrophobic mismatch between proteins and lipids in membranes. Biochim Biophys Acta 1998; 1376:401–415.

Koenig BW, Strey HH, Gawrisch K. Membrane lateral compressibility determined by NMR and x-ray diffraction: effect of acyl chain polyunsaturation. Biophys J 1997; 73:1954–1966.

Kuwahara Y, Yanagishita T, Konno N, Katagiri T. Changes in microsomal membrane phospholipids and fatty acids and in activities of membrane-bound enzyme in diabetic rat heart. Basic Res Cardiol 1997; 92:214–222.

Lee AG. How lipids interact with an intrinsic membrane protein: the case of the calcium pump. Biochim Biophys Acta 1998; 1376:381–390.

Li L, Zheng LX, Yang FY. Effect of propensity of hexagonal II phase formation on the activity of mitochondrial ubiquinol-cytochrome c reductase and H(+)-ATPase. Chem Phys Lipids 1995; 76:135–144.

Litman BJ, Lewis EN, Levin IW. Packing characteristics of highly unsaturated bilayer lipids: Raman spectroscopic studies of multilamellar phosphatidylcholine dispersions. Biochemistry 1991; 30:313–319.

Litman BJ, Mitchell DC. Rhodopsin structure and function. In: Lee AG, ed, Biomembranes, Volume 2A Jai, Greenwich, CT, 1996 , pp. 1–32.

Litman BJ, Mitchell DC. A role for phospholipid polyunsaturation in modulating membrane protein function. Lipids 1996; 31:S193–S197.

Litman BJ, Niu SL, Polozova A, Mitchell DC. The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: Visual transduction. Mol Cell Neuorsci, In Press, 2001.

Matthews PL, Bartlett E, Ananthanarayanan VS, Keough KM. Reconstitution of rabbit sarcoplasmic reticulum calcium ATPase in a series of phosphatidylcholines containing a saturated and an unsaturated chain: suggestion of an optimal lipid environment. Biochem Cell Biol 1993; 71:381–389.

McElhaney RN. The structure and function of the Acholeplasma laidlawii plasma membrane. Biochim Biophys Acta 1984; 779:1–42.

Mitchell DC, Straume M, Miller JL, Litman BJ. Modulation of metarhodopsin formation by cholesterol-induced ordering of bilayer lipids. Biochemistry 1990; 29:9143–9149.

Mitchell DC, Straume M, Litman BJ. Role of sn-1-saturated, sn-2-polyunsaturated phospholipids in control of membrane receptor conformational equilibrium: effects of cholesterol and acyl chain unsaturation on the metarhodopsin I in equilibrium with metarhodopsin II equilibrium. Biochemistry 1992; 31:662–670.

Mitchell DC, Litman BJ. Molecular order and dynamics in bilayers consisting of highly polyunsaturated phospholipids. Biophys J 1998; 74:879–891.

Mitchell DC, Litman BJ. Effect of cholesterol on molecular order and dynamics in highly polyunsaturated phospholipid bilayers. Biophys J 1998; 75:896–908.

Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentration and mortality: a quantitative review of primary prevention trials. Br Med J 1990; 301:309–314.

Nabekura J, Noguchi K, Witt MR, Nielsen M., Akaike N. Functional modulation of human recombinant gamma-aminobutyric acid type A receptor by docosahexaenoic acid. J Biol Chem 1998; 273:11,056–11,061.

Needham D, Nunn RS. Elastic deformation and failure of lipid bilayer membranes containing cholesterol. Biophys J 1990; 58:997–1009.

Neuringer M, Connor WE, Van Petten C, Barstad L. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. J Clin Invest 1984; 73:272–276.

Niebylski CD, Salem N Jr. A calorimetric investigation of a series of mixed-chain polyunsaturated phosphatidylcholines: effect of sn-2 chain length and degree of unsaturation. Biophys J 1994; 67:2387–2393.

O'Brien DF, Costa LF, Ott RA. Photochemical functionality of rhodopsin-phospholipid recombinant membranes. Biochemistry 1977; 16:1295–1303.

Olbrich K, Rawicz W, Needham D, Evans E. Water permeability and mechanical strength of polyunsaturated lipid bilayers. Biophys J 2000; 79:321–327.

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000; 289:739–745.

Poling JS, Vicini S, Rogawski MA, Salem N Jr. Docosahexaenoic acid block of neuronal voltage-gated K^+ channels: subunit selective antagonism by zinc. Neuropharmacology 1996; 35:969–982.

Polozova A, Litman BJ. Cholesterol dependent recruitment of di22:6 PC by a G protein-coupled receptor into lateral domains. Biophys J 2000; 79(5):2632–2643.

Rawicz W, Olbrich KC, McIntosh T, Needham D, Evans E. Effect of chain length and unsaturation on elasticity of lipid bilayers. Biophys J 2000; 79:328–339.

Salem N Jr. Omega-3 fatty acids: molecular and biochemical aspects. In: Spiller GA, Scala J, eds., New Protective Roles for Selected Nutrients. Alan R. Liss, New York, 1989, pp. 109–228.

Schroeder F, Woodford JK, Kavecansky J, Wood WG, Joiner, C. Cholesterol domains in biological membranes. Mol Membr Biol 1995; 12:113–119.

Separovic F, Gawrisch K. Effect of unsaturation on the chain order of phosphatidylcholines in a dioleoylphosphatidylethanolamine matrix. Biophys J 1996; 71:274–282.

Skuladottir GV, Schioth HB, Gudbjarnason, S. Polyunsaturated fatty acids in heart muscle and alpha 1-adrenoceptor binding properties. Biochim Biophys Acta 1993; 1178:49–54.

Slater SJ, Kelly MB, Taddeo FJ, Ho C, Rubin E, Stubbs CD. The modulation of protein kinase C activity by membrane lipid bilayer structure. J Biol Chem 1994; 269:4866–4871.

Smaby JM, Momsen MM, Brockman HL, Brown RE. Phosphatidylcholine acyl unsaturation modulates the decrease in interfacial elasticity induced by cholesterol. Biophys J 1997; 73:1492–1505.

Stillwell W, Ehringer WD, Dumaual AC, Wassall SR. Cholesterol condensation of alpha-linolenic and gamma-linolenic acid-containing phosphatidylcholine monolayers and bilayers. Biochim Biophys Acta 1994; 1214:131–136.

Stinson AM, Wiegand RD, Anderson RE. Fatty acid and molecular species compositions of phospholipids and diacylglycerols. Exp Eye Res 1991; 52:213–218.

Stinson AM, Wiegand RD, and Anderson RE. Recycling of docosahexaenoic acid in rat retinas during n-3 fatty acid deficiency. J Lipid Res 1991; 32:2009–2017.

Straume M, Litman BJ. Influence of cholesterol on equilibrium and dynamic bilayer structure of unsaturated acyl chain phosphatidylcholine vesicles as determined from higher order analysis of fluorescence anisotropy decay. Biochemistry 1987; 26:5121–5126.

Straume M, Litman BJ. Equilibrium and dynamic bilayer structural properties of unsaturated acyl chain phosphatidylcholine–cholesterol–rhodopsin recombinant vesicles and rod outer segment disk membranes as determined from higher order analysis of fluorescence anisotropy decay. Biochemistry 1988; 27:7723–7733.

Tanskanen A, Vartianinen E, Tuomilehto J, Viinamaki H, Lehtonen J, Puska P. High serum cholesterol and risk of suicide. Am J Psychiatry 2000; 157:648–650.

van Ginkel G, van Langen H, Levine YK. The membrane fluidity concept revisited by polarized fluorescence spectroscopy on different model membranes containing unsaturated lipids and sterols. Biochimie 1989; 71:23–32.

Wiedmann TS, Pates RD, Beach JM, Salmon A, Brown MF. Lipid–protein interactions mediate the photochemical function of rhodopsin. Biochemistry 1988; 27:6469–6474.

Zerouga M, Jenski LJ, Stillwell W. Comparison of phosphatidylcholines containing one or two docosahexaenoic acyl chains on properties of phospholipid monolayers and bilayers. Biochim Biophys Acta 1995; 1236:266–272.