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CHOLESTEROL LEVELS EXPLAIN INVERSE COMPENSATION OF MEMBRANE ORDER IN BRUSH BORDER BUT NOT HOMEOVISCOUS ADAPTATION IN BASOLATERAL MEMBRANES FROM THE INTESTINAL EPITHELIA OF RAINBOW TROUT

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Summary

The role of cholesterol in the thermal adaptation of biological membranes is explored. Physical and chemical responses of membranes to acclimation temperature were evaluated using plasma membrane domains (basolateral and brush border) prepared from intestinal epithelia of 5- and 20 °C-acclimated rainbow trout (*Oncorhynchus mykiss*). Basolateral membranes (BLMs) exhibit perfect homeoviscous efficacy (indicated by fluorescence depolarization using 1,6-diphenyl-1,3,5-hexatriene), although cholesterol content does not change with acclimation temperature (molar ratios of cholesterol to phospholipid are 0.23± 0.01 from 5 °C-acclimated fish and 0.25±0.02 from 20°C-acclimated fish; mean ± S.E.M.). Reductions (greater than 30 %) in each of the two major saturated fatty acids (16:0 and 18:0), and a 42 % increase in the polyunsaturate 22:6 ($n-3$) are found in BLMs from fish acclimated to 5 °C compared with membranes from warm-acclimated animals, suggesting that the phospholipid acyl chain composition determines the physical properties of BLMs. In marked contrast, brush-border membranes (BBMs) display opposite trends. BBMs from 5 °C-acclimated fish are more ordered than BBMs from 20 °C-acclimated fish (inverse compensation). Cholesterol content expressed relative to protein or relative to total polar lipid (phospholipid plus glycolipid) is significantly higher in cold- than in warm-acclimated fish, and nearly so (P=0.15) relative to phospholipid (0.31±0.03 in 5 °C-acclimated animals and 0.25±0.02 in 20 °C-acclimated animals). Only minor changes in the acyl composition of BBMs are induced by temperature acclimation. These results suggest that bile, a constituent of the apical microenvironment, may impose unusual requirements for membrane order and/or stability in the brush border.

Key words: brush-border, basolateral membranes, cholesterol, temperature acclimation, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Preserving the function of biological membranes is a particular challenge for poikilothermic organisms, whose body temperatures vary with ambient temperature. Membrane integrity must be maintained for membranes to operate as a selective barrier. In addition, the physical and chemical environment of the membrane must be regulated to allow continuity of activity of membrane-associated proteins (e.g. receptors, enzymes, channels). Homeoviscous adaptation, the maintenance of membrane fluidity, has been observed in diverse taxa including bacteria (Sinensky, 1974), plants (Raison *et al.* 1982) and animals (Behan-Martin *et al.* 1993). Homeoviscous adaptation is undoubtedly an important adaptive response of many poikilotherms.

Restructuring of membrane phospholipids is a common mechanism to ensure the stability of membrane function at different temperatures in poikilotherms. Changes in the relative distribution of phospholipid classes (e.g. phosphatidylcholine, phosphatidylethanolamine) and phospholipid molecular species (e.g. 1-palmitoyl 2-docosahexaenoyl phosphatidylcholine, 1-palmitoyl 2-oleoyl phosphatidylcholine) have been extensively studied as potential mechanisms of homeoviscous adaptation (reviewed by Cossins and Sinensky, 1984; Hazel and Williams, 1990). Relatively little attention has been given to the potential role of cholesterol, a common neutral lipid of plasma membranes in animals, in the thermal adaptation of biological membranes. It is well established that cholesterol typically imparts order to fluid-phase membranes (Yeagle, 1987). An increase in plasma membrane cholesterol should offset, at least in part, the fluidizing effect of elevated body temperatures, while a decrease in cholesterol content at cold body temperatures should enhance the fluid nature of the membrane.

Results from previous studies documenting the effects of temperature on the cholesterol content of biological

membranes suggest that, at higher growth temperatures, there is an increase in membrane cholesterol (e.g. Wodtke, 1978; Downer and Kallapur, 1981; Chang and Roots, 1989; Sørenson, 1993). These results are to be expected if the ordering effect of cholesterol were to play a role in homeoviscous adaptation. However, much of the earlier work was performed on intracellular membranes (Wodtke, 1978; Downer and Kallapur, 1981; Chang and Roots, 1989). Recently, Lange *et al.* (1989) have convincingly demonstrated that as much as 90 % of membrane-associated cholesterol is localized in plasma membranes and that cholesterol previously attributed to intracellular membranes is probably a reflection of contamination by plasma membrane fragments. These results demonstrate the importance of re-examining the role of cholesterol modulation in thermal acclimation using purified plasma membranes.

The major objective of this study was to evaluate the role of cholesterol in the thermal adaptation of plasma membranes. We have determined whether cholesterol content in plasma membranes is modulated with acclimation temperature using two distinct plasma membrane domains (the basolateral and brush-border membranes) of rainbow trout enterocytes. We have studied both plasma membrane domains because they differ in physiological function as well as in chemical composition and physical properties (Simons and van Meer, 1988).

Our results demonstrate that cholesterol modulation occurs only in the brush-border membrane and in a way that explains inverse compensation of membrane order. Changes in acyl chain composition explain homeoviscous adapation in the basolateral domain. We suggest that the chemical and physical responses of membranes to temperature challenges may vary with membrane function and with the microenvironment to which the membrane is exposed.

Materials and methods

Animals

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), were obtained from the Alchesay National Fish Hatchery in Whiteriver, Arizona, in the spring of 1993. Fish were brought to Arizona State University and acclimated to either 5 °C or 20 °C for a minimum of 4 weeks before use (12 h:12 h L:D photoperiod). Daily feeding consisted of pelleted trout chow from Glencoe Mills.

Membrane preparations

Brush-border (BBMs) and basolateral (BLMs) membranes were prepared from the intestinal epithelia (posterior from the pyloric caecal region) using a combination of differential and density gradient centrifugation (see Fig. 1). The method we describe below represents a significant departure from published methods employing divalent cation precipitation (e.g. Lee and Cossins, 1990), which proved unsuccessful in our hands. Instead, the initial preparative steps are based on those used in Hazel *et al.* (1992) and the subsequent steps were developed empirically.

Intestines were removed, rinsed in 150 mmol l^{-1} NaCl, 25 mmol l^{-1} Tris–HCl, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol (DTT) (pH 7.4 at 25 °C) and cut longitudinally. Intestines were pinned to expose the luminal surfaces and rinsed thoroughly. Epithelial cells were obtained by scraping with a glass coverslip. Cells were homogenized in 4 volumes of homogenization medium (250 mmol l^{-1} sucrose, 25 mmol l^{-1} Tris–HCl, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} benzamidine, pH 7.4 at 25 °C) containing DNAase (3 μg per 4 g tissue) using a Potter–Elvehjem homogenizer (six passes, 1000 revs min^{-1}). Epithelial scrapings from a minimum of four fish corresponding to about 4 g (wet mass) of tissue were pooled to constitute single preparations of BBMs and BLMs.

Homogenates were layered over 41 % sucrose (in 25 mmol l^{-1} Tris–HCl) and centrifuged for 30 min at 23 000 g_{av} in a fixed-angle rotor (Beckman JA-20). Material at the 41 % sucrose interface (I1-1) and material that pelleted through the sucrose (P1-1) were collected. (Typically a bolus of mucus

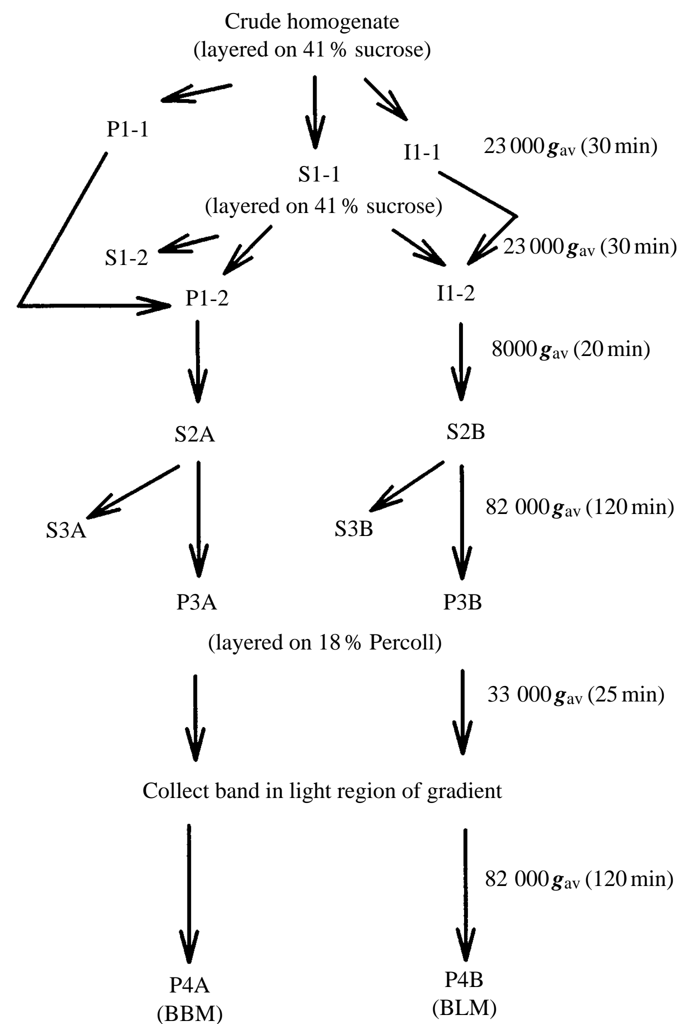

Fig. 1. Simultaneous preparation of basolateral (BLM) and brush-border (BBM) membranes using differential and density gradient centrifugation. P, pellet, S, supernatant, I, interface.

formed in homogenates from warm-acclimated fish and this was discarded.) Supernatants (S1-1) were collected and centrifuged again for 30 min at 23 000 g_{av} over 41 % sucrose, and the interface (I1-2) and pelleting (P1-2) materials were again collected.

Interfaces (combined I1-1 and I1-2) and pellets (combined P1-1 and P1-2) were passed through a loose-fitting, 7 ml Ten Broeck ground-glass homogenizer and resuspended in a volume of homogenization medium (see above) four times their volume and then centrifuged for 20 min at 8000 g_{av}. The supernatants (S2A from pellets, S2B from interfaces) were collected and centrifuged for 120 min at 82 000 g_{av} to form pellets P3A (from supernatant 2A) and P3B (from supernatant 2B).

These pellets (P3A and P3B) were resuspended in 1 ml of homogenization medium and homogenized gently in 2 ml Ten Broeck ground-glass homogenizers. These homogenates were then applied to a solution of 18 % Percoll (250 mmol l^{-1} sucrose, 25 mmol l^{-1} Tris–HCl, 1 mmol l^{-1} EDTA, pH 7.4 at 25 °C) and a self-generated gradient was formed using a Beckman 50.2 Ti fixed-angle rotor (33 000 g_{av}, 25 min at full speed). In each case, single bands were apparent in the less dense region of the gradients. These bands were collected by upward displacement of the gradient, diluted to a volume about four times that of the band volume in 150 mmol l^{-1} NaCl, 25 mmol l^{-1} Tris–HCl, 1 mmol l^{-1} EDTA (pH 7.4 at 25 °C), and centrifuged for 120 min at 82 000 g_{av}. Finally, the thin sheets of membranous material residing above the Percoll pellets (P4A corresponding to BBMs and P4B corresponding to BLMs) were collected, made up to about 0.5 ml with 25 mmol l^{-1} Tris-HCl (pH 7.4 at 25 °C) and homogenized in 2 ml Ten Broeck ground-glass homogenizers before freezing (−70 °C).

Organelle marker enzymes were assayed to establish relative enrichment and purity of membranes. Enzymes used as markers were as follows: leucine aminopeptidase for brush-border membranes (Hasse *et al.* 1978), Na^+/K^+-ATPase for basolateral membranes (Barnett, 1970), succinate cytochrome *c* reductase for mitochondria (King, 1967), catalase for peroxisomes (Beers and Sizer, 1952), glucose-6-phosphatase for endoplasmic reticulum (Aronson and Touster, 1974) and β-*N*-acetylhexosaminidase for lysosomes (Levvy and Conchie, 1966; Stirling, 1984).

Fluorescence depolarization methods

Steady-state fluorescence polarization values were determined for BBMs and BLMs using the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Tris–HCl (25 mmol l^{-1}, pH 7.4) was used as resuspension buffer. A Perkin–Elmer LS-50B luminescence spectrometer monitored fluorescence with excitation and emission monochrometers set at 356 nm and 430 nm, respectively. Initially, polarization values were measured for BBMs and BLMs in 1 °C increments to ensure that no phase transitions occurred. Since no phase transitions were observed in the temperature range 5–25 °C for membranes from either acclimation group, subsequent polarization values were measured at 2 °C intervals.

Representative regressions for BBMs or BLMs were estimated using the following procedure. Regression equations were established for polarization values measured at every 1 or 2 °C described above for each membrane sample. From the individual regression equations, polarization values were calculated at 5 °C temperature increments (25, 20, 15, 10, 5) for each sample. Means and standard deviations of these polarization values were plotted and analyzed statistically as described below (N=4 separate membrane preparations for both BBMs and BLMs at each acclimation temperature).

Analysis of phospholipid fatty acyl composition

Total lipid extracts were prepared from plasma membrane preparations according to Bligh and Dyer (1959). Lipid extracts were methylated using 3 mol l^{-1} methanolic HCl (Christie, 1982). Fatty acid methyl esters were separated by capillary gas chromatography (Omegawax 320, 30 m, isothermal at 190 °C, Hewlett Packard 5840A chromatograph, N_2 carrier gas). Peak identification was accomplished by reference to commercial standards. Peaks were considered for analysis if they constituted 1 % or more of total fatty acid methyl esters.

Cholesterol, phospholipid and protein determinations

Cholesterol contents of biological membranes were determined by a fluorometric/enzymatic assay that utilizes cholesterol oxidase (Crockett and Hazel, 1995). Phospholipid was quantified as total phosphate (from acid hydrolysis of phospholipids) using the method of Rouser *et al.* (1970). Protein was assayed using the bicinchoninic acid method (Smith *et al.* 1985).

Glycolipid analyses

Glycolipid contents in BLMs and BBMs were analyzed according to the procedure described in Kushwaha and Kates (1981) using glucose as a standard. For purposes of calculation, we assumed a 1:1 molar ratio of hexose to glycolipid.

Statistical analyses

Pairwise comparisons were analyzed using two-tailed Student's *t*-test. Ratios were transformed (arcsine). Comparisons of fluidity were analyzed by analysis of covariance (ANCOVA) using Systat (Evanston, Illinois, USA).

Chemicals

All substrates and most enzymes were from Sigma Chemical Co. (St Louis, MO, USA). Cholesterol oxidase was from Beckman Instruments (Carlsbad, CA, USA). DPH was from Molecular Probes (Eugene, OR, USA). Organic solvents were washed if necessary and redistilled before use.

Results

Brush-border and basolateral membrane enrichment

BLMs are enriched 10- to 11-fold in the basolateral marker enzyme Na^+/K^+-ATPase and BBMs are enriched five- to

Table 1. *Marker enzyme enrichment factors for plasma membranes and intracellular organelles*

Membrane	Temperature (°C)	LAP (BBM)	Na^+/K^+-ATPase (BLM)	SCCRed (M)	CAT (P)	G6Pase (ER)	AHEX (L)
BLM	20	1.7±0.1	10.0±0.9				
BLM	20, 5	(6)		<1 (3)	<1 (3)	2 (3)	<1(3)
BLM	5	1.9±0.2	11.4±1.7				
BBM	20	6.5±0.8	4.4±0.4				
BBM	20, 5	(6)		<1 (2)	<1 (2)	2 (2)	<1(3)
BBM	5	4.7±0.4	1.7±0.5				

Enrichment factors are calculated as protein specific activity of membrane *versus* protein specific activity of crude homogenate (*N*).

Acclimation temperatures are given beside membrane.

Abbreviations are as follows for organelles and marker enzyme activity: BLM, basolateral membranes; BBM, brush-border membranes; M, mitochondria; P, peroxisomes; ER, endoplasmic reticulum; L, lysosomes; LAP, leucine aminopeptidase; SCCRed, succinate cytochrome *c* reductase; CAT, catalase; G6Pase, glucose-6-phosphatase; AHEX, β-*N*-acetylhexosaminidase.

Values are mean ± S.E.M.

sixfold in the apical marker leucine aminopeptidase (LAP) (Table 1). Enrichment factors are low (less than 1 in most cases) for marker activities indicative of other organelles (mitochondria, peroxisomes, endoplasmic reticulum and lysosomes).

Recovery experiments were also performed to ensure that enrichment factors reflected a true increase in purity and not simply an apparent increase in purity. Protein content, LAP and Na^+/K^+-ATPase activities were measured in homogenates and all fractions collected after the first centrifugation step. Recoveries of both protein and LAP activity in various fractions summed to 105 % of that in the crude homogenate (N=4). However, recovery of Na^+/K^+-ATPase activity averaged 148 % of that in the homogenate, probably reflecting loss of activity in the homogenate due to endogenous protease activity only partially offset by benzamidine. This suggests that Na^+/K^+-ATPase measured in the final membrane fractions overestimates enrichment by 1.5-fold. If this is the case, enrichment factors for BLMs would be sevenfold (instead of 10- to 11-fold), comparable to BBM enrichments, and Na^+/K^+-ATPase activity in the BBMs (reflecting apparent contamination of BBMs by BLMs) would also be reduced. These results, in combination with differences in the chemical and physical properties of BLMs and BBMs described below, clearly indicate the isolation of distinct plasma membrane domains.

Membrane order changes with acclimation temperature in BLMs and BBMs

Membrane order, as indicated by DPH fluorescence depolarization, is modulated with acclimation temperature in an opposite manner in BLMs and BBMs from trout intestinal epithelia (Fig. 2). BLMs from cold-acclimated animals are less ordered (lower fluorescence polarization values) than BLMs from warm-acclimated animals (analysis of covariance, ANCOVA, P<0.001) and homeoviscous efficacy is perfect (the order of membranes from 20 °C-acclimated fish measured at 20 °C matches the order of membranes from 5 °C-acclimated fish measured at 5 °C, P=0.96). In contrast, inverse compensation is observed in BBMs. At all temperatures measured, BBMs from cold-acclimated fish are more ordered than BBMs from warm-acclimated fish (ANCOVA, P<0.001).

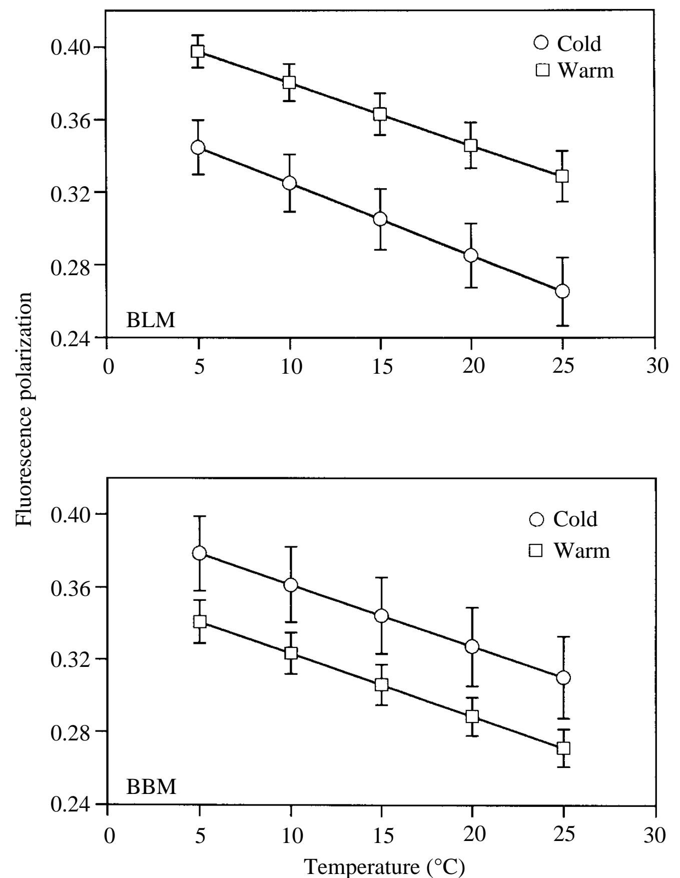

Fig. 2. Steady-state fluorescence polarization values for the fluorescent probe DPH in BLMs and BBMs from warm- (20 °C) and cold- (5 °C) acclimated fish. Each symbol represents the mean ± S.D. (N=4).

Table 2. *Percentage composition of phospholipid acyl chains from basolateral membranes and brush-border membranes of trout intestinal epithelia*

	Basolateral membranes		Brush-border membranes	
Fatty acid	20 °C acclimation	5 °C acclimation	20 °C acclimation	5 °C acclimation
14:0	1.38±0.10	1.47±0.11	1.73±0.17	2.08±0.06
16:0	37.3±2.17	24.1±1.25**	46.9±3.51	42.6±1.51
16:1	1.68±0.06	2.14±0.12*	1.76±0.12	2.56±0.19*
18:0	8.78±0.42	5.97±0.03***	9.65±1.21	7.50±0.21
18:1	12.0±0.65	11.8±0.32	10.9±2.10	10.4±0.68
18:2	2.86±0.15	2.92±0.06	2.46±0.35	3.09±0.23
20:0	1.26±0.13	2.64±0.35*	1.01±0.50	1.92±0.11
20:2	1.01±0.12	1.30±0.09	0	1.19±0.16
20:3	0.69±0.05	1.11±0.10**	0	0
20:4	1.62±0.06	2.80±0.27**	0.70±0.39	1.84±0.18*
20:5	3.78±0.16	4.50±0.26	2.84±0.27	2.44±0.17
22:4	1.74±0.27	2.62±0.25	3.81±1.06	1.65±0.40
22:5	1.26±0.09	2.62±0.25**	0.36±0.29	1.39±0.16*
22:6	21.5±1.65	30.5±2.28*	13.0±2.80	16.0±1.82
24:1	1.40±0.14	1.74±0.30	1.15±0.11	1.67±0.22

Values shown are means ± S.E.M. expressed as a percentage of total phospholipid fatty acyl chains.
Fatty acids shown are those constituting more than 1% of the total. N=4 in all cases (except BBMs at 20 °C, N=3).
*P<0.05; **P<0.01; ***P<0.001.

Temperature sensitivities of membrane order are similar for membranes from warm- and cold-acclimated fish since the slopes calculated for polarization values *versus* assay temperatures are homogeneous within a particular membrane domain (P=0.70 for BLMs, P=0.92 for BBMs). Temperature sensitivities are also comparable for BLMs and BBMs (P=0.33).

Composition of phospholipid fatty acyl chains

BBMs contain more saturated fatty acyl chains (particularly high levels of 16:0 and low levels of 22:6 fatty acids) than BLMs (Table 2). A comparison of phospholipid fatty acyl chain compositions from BLMs and BBMs reveals different compositional responses to growth temperature. Levels of eight fatty acids are modified with acclimation temperature in BLMs, whereas compositional changes are apparent for only three fatty acids in BBMs. The magnitude of the change in fatty acid composition is also greater in BLMs. Contents of the three fatty acid species representing the majority of the total pool, 16:0, 18:0 and 22:6, change significantly with acclimation temperature in BLMs but are unaffected by acclimation temperature in BBMs.

Compositional changes in BLMs include a reduction in the content of saturated fatty acids (e.g. 16:0 and 18:0) and an increase in the content of unsaturated fatty acids (e.g. 22:6, 20:3, 20:4 and 22:5) at low acclimation temperature. These trends are reflected in differences in unsaturation indices (UI, the average number of double bonds per fatty acyl chain) between acclimation temperatures (Fig. 3). UI is nearly 1.4-fold higher (P<0.05) for BLMs from cold-acclimated animals than for BLMs from warm-acclimated animals, whereas differences in UI between acclimation temperatures are not

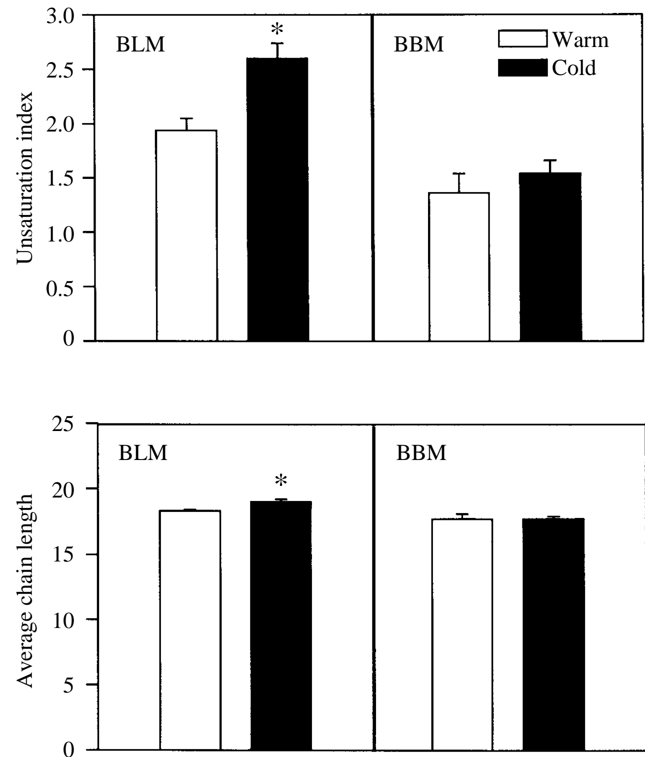

Fig. 3. Unsaturation index and average chain length of phospholipid fatty acids in BLMs and BBMs from warm- (20 °C) and cold- (5 °C) acclimated trout (N=4 in all cases, except BBMs at 20 °C, where N=3). Data presented as means + S.E.M. *Significantly different from the value for warm-acclimated fish, P<0.05.

significantly different for BBMs. Average chain lengths are sensitive to changes in acclimation temperature in membranes from the basolateral domain only. Acyl chain length is greater in BLMs from cold- than from warm-acclimated fish (Fig. 3).

Cholesterol content in BLMs and BBMs

Cholesterol content relative to the contents of other membrane constituents varies with acclimation temperature only in the BBMs (Fig. 4). Cholesterol content is greater in BBMs from cold- than from warm-acclimated animals when expressed relative to protein ($P<0.02$), or relative to total polar lipid ($P<0.03$) content. Though not significant ($P=0.15$), cholesterol:phospholipid molar ratios in BBMs appear to be higher in cold- than in warm-acclimated trout (0.31±0.03 in the cold-acclimated fish and 0.25±0.02 in the warm-acclimated fish, mean ± S.E.M.).

In contrast, cholesterol content does not change with acclimation temperature in BLMs. Cholesterol content is not altered with acclimation temperature when viewed relative to protein, phospholipid or total polar lipid (Fig. 4) content. Molar ratios of cholesterol:phospholipid are 0.25±0.02 (warm-acclimated) and 0.23±0.01 (cold-acclimated).

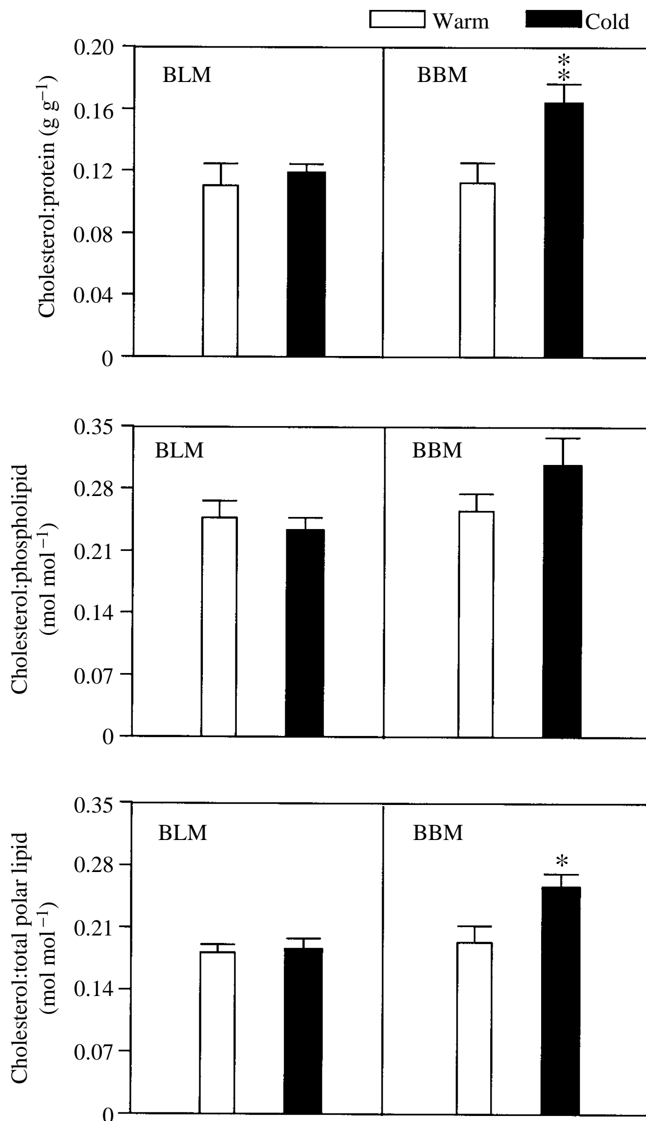

Fig. 4. Cholesterol content normalized to protein, phospholipid or total polar lipid content in BLMs and BBMs from warm- (20 °C) and cold- (5 °C) acclimated fish. Mass ratios were calculated for cholesterol:protein, whereas molar ratios were calculated for cholesterol:phospholipid and cholesterol:total polar lipid. $N=6$ for cholesterol relative to either protein or phospholipid; $N=4$ for cholesterol relative to total polar lipid. Data presented as means + S.E.M. *Significantly different from the value for warm-acclimated fish, $P<0.05$, ** $P<0.01$.

Glycolipid content in BLMs and BBMs

Lipid compositional analyses indicate that the relative proportion of glycolipid does not change with acclimation temperature in either membrane; however, a trend towards higher glycolipid content in BLMs from warm- compared with cold-acclimated animals emerges ($P=0.08$) (Table 3).

Discussion

Cholesterol contents of plasma membranes may be compared when normalized to the content of other membrane constituents. Molar ratios of cholesterol:phospholipid are often used, but Simons and van Meer (1988) have emphasized the importance of including the glycolipid component of the membrane in these estimates. Not only can the glycolipid component be a significant fraction of the total membrane lipid, but the glycolipid content varies with plasma membrane domain. We have examined cholesterol content in two plasma membrane domains from the intestinal epithelia of temperature-acclimated trout. We have expressed membrane cholesterol content by normalizing to membrane protein, phospholipid and total polar lipid (phospholipid and glycolipid) content.

Homeoviscous adaptation in BLMs is explained by changes in phospholipid acyl chain composition

Perfect homeoviscous efficacy is observed in BLMs from rainbow trout intestinal epithelia. Homeoviscous efficacy (75 %) has been demonstrated previously in BLMs from the intestinal epithelia of a vertebrate poikilotherm (carp) (Lee and Cossins, 1990). Presumably this adaptation permits continuity

Table 3. *Relative distribution of lipids in basolateral and brush-border membranes of trout intestinal epithelia*

	Basolateral membranes (mol%)		Brush-border membranes (mol%)	
	20 °C	5 °C	20 °C	5 °C
GL	28.1±3.3	20.0±1.9	19.6±1.9	18.7±2.9
PL	56.6±3.0	64.3±2.1	64.2±2.3	60.9±2.6
C	15.3±0.6	15.6±0.7	16.1±1.1	20.4±0.8

GL, glycolipid; PL, phospholipid; C, cholesterol.
Values represent mean ± S.E.M., $N=4$.

of membrane and membrane-associated functions by preserving membrane physical properties.

BLMs modulate phospholipid acyl chain composition and perhaps glycolipid content, but not cholesterol levels, in response to acclimation temperature. Homeoviscous adaptation in BLMs is achieved, at least in part, by changes in the acyl chain composition of the phospholipids. Adjustments in acyl chain composition to acclimation temperature are not unique to BLMs from trout. Increases in the degree of unsaturation of phospholipid acyl chains have been observed in BLMs from carp raised at 10 °C compared with membranes from animals raised at 30 °C (Lee and Cossins, 1990).

Fluidity and cholesterol:phospholipid ratios may be preserved in BLMs in order to maintain the functionality of the integral membrane protein Na^+/K^+-ATPase that maintains the Na^+ and K^+ gradients. Cholesterol:phospholipid molar ratios of 0.4–0.5 yield maximal activity of Na^+/K^+-ATPase in plasma membranes from mammalian erythrocytes and kidney (Yeagle, 1983; Yeagle *et al.* 1988). Chemical and/or physical constancy may be necessary to preserve sufficient function of Na^+/K^+-ATPase in intestinal BLMs from rainbow trout.

Membrane order in BBMs is inversely compensated as explained by levels of cholesterol

BBMs from cold-acclimated fish are more ordered at all temperatures than comparable membranes from warm-acclimated fish (inverse compensation). Previous studies employing BBMs from cold- and warm-acclimated carp and roach reported no compensation (similar fluidities at common measurement temperatures) (Lee and Cossins, 1990; Schwarzbaum *et al.* 1992). This means that, when compared at physiological temperatures, membranes from cold-acclimated fish are more ordered than membranes from warm-acclimated fish. These results coupled with our own observations suggest the intriguing possibility that brush-border membranes from a variety of cold-acclimated or cold-adapted poikilothermic animals (at least when compared at physiological temperatures) may be more ordered than counterpart membranes from organisms living at warmer body temperatures.

True inverse compensation in membrane order as reported in this study (a higher order in membranes of cold- than in warm-acclimated animals at all assay temperatures) is a relatively rare phenomenon. Dahlhoff and Somero (1993) recently showed that mitochondrial membranes from the hepatopancreas of the cold-acclimated red abalone, *Haliotis rufesens*, were more ordered than similar membranes from animals acclimated to warmer temperatures. This result was not observed in other abalone species examined.

Changes in membrane order in the BBM domain are largely predicted by cholesterol levels because acyl chain composition does not change with acclimation temperature. Similarly, phospholipid acyl chain composition reported as either unsaturation index or saturation ratio does not change with acclimation temperature in carp (Lee and Cossins, 1990). In the present study, the acclimation group having the highest cholesterol content (cold-acclimated) also has the most ordered membranes. Although we cannot exclude the possibility that changes in phospholipid class (polar head group) may occur in BBMs, changes in the level of cholesterol probably represent the most significant compositional adjustment to acclimation temperature. Our observation that one membrane domain changes its cholesterol content (brush border) while another does not (basolateral membranes) suggests that the acquisition of enterocyte plasma membrane cholesterol is a regulated process and not a passive consequence of differences in cholesterol uptake or biosynthesis. Adjusting cholesterol content in BBMs may be a more efficient means of modulating physical properties than changing acyl chain composition. Little expenditure of energy may be required for cholesterol movement within the cell.

Why do BLMs and BBMs adjust membrane order in an opposite manner?

It is worthwhile considering why basolateral and brush-border domains modulate physical properties in opposite ways in response to acclimation temperature (homeoviscous adaptation *versus* inverse compensation). Our results, coupled with those reported by Lee and Cossins (1990) and Schwarzbaum *et al.* (1992), suggest that homeoviscous adaptation may be a general trend for BLMs but that BBMs from cold-acclimated animals are more ordered, at least at physiological temperatures, than BBMs from warm-acclimated animals. These observations pose an interesting question: why do these physical characteristics of the two membrane domains vary with acclimation temperature in dissimilar ways?

One possible explanation is that there may be differences in the microenvironments of the BLMs and the BBMs that require dissimilar physical responses on the part of the membranes. Apical membranes from mammalian epithelia (intestine and kidney) are typically more ordered than their basolateral counterparts, presumably to provide stability to the apical domain, which faces the more variable external environment (Simons and van Meer, 1988). Perhaps the most likely scenario to explain the present results is that bile, a major constituent of the BBM microenvironment (the gut lumen), is in part responsible for the trends we have observed with the BBMs. The detergent action of bile is potentially perturbing to membranes (Schmucker *et al.* 1990; Krähenbühl *et al.* 1994). Several characteristics of biliary production and properties, if they vary with acclimation temperature, may necessitate specific adjustments to membrane physical properties to compensate for bile action. First, the concentration of some component of the bile (e.g. bile salts) may change with acclimation temperature. Schölmerich *et al.* (1984) have shown that the extent of membrane damage varies with bile salt composition. Second, bile salts may readily incorporate, at least transiently, into brush-border membranes. Kamp and Hamilton (1993) have shown that bile acids will undergo transbilayer movement in small unilamellar vesicles. These temporary changes are likely to affect the physical state of the membrane. Membrane

chemical and physical properties may be adjusted to offset the effects of transient bile components. Finally, bile secretion rates and/or residence times may change with acclimation temperature and so affect the concentration of bile in the lumen.

An alternative explanation for the different physical responses to acclimation temperature again involves the distinct microenvironments of the BLMs and BBMs. Digestive activity, and specifically lipolysis, may be enhanced at low temperatures to maintain digestion efficiencies (Hazel and Prosser, 1974). A more ordered membrane may be required at low temperatures to offset perturbation due to endogenous lipases.

Membrane specificity of responses

The role(s) of cholesterol in thermal adaptation of plasma membranes is (are) membrane-specific. All of the three possible outcomes for cholesterol modulation with respect to growth or acclimation temperature have been observed. First, cholesterol content remains constant (BLMs, present study; intestinal plasma membranes, Miller *et al.* 1976). Second, cholesterol levels vary and are higher at elevated body temperatures (the response we would expect if cholesterol were to be involved in offsetting the effect of temperature on membrane order). This response has been observed in plasma membranes from erythrocytes (Sørenson, 1993) as well as from hepatic, renal and gill tissues (J. C. Robertson and J. R. Hazel, in preparation). In addition, cholesterol contents in plasma membranes of even more warm-bodied organisms, such as mammals, are typically higher than those of cold-bodied ectotherms (for mammals, see Simons and van Meer, 1988). Finally, cholesterol content increases at cold body temperature relative to levels at warm body temperature (BBMs, present study). Clearly no single response of cholesterol is apparent, which indicates a complexity of roles for cholesterol in thermal adaptation.

Inverse compensation (BBM, present study; hepatopancreatic mitochondrial membranes, Dahlhoff and Somero, 1993) and a lack of compensation in membrane order (BBM, Lee and Cossins, 1990; Schwarzbaum *et al.* 1992) point to the likelihood that other physical properties besides membrane order may be preserved during temperature acclimation. Although homeoviscous adaptation occurs in different biological membranes from a variety of taxonomic groups, it is not the sole mechanism involved in ensuring the continuance of membrane function. For example, the proximity to the H_{II} phase transition may be preserved to enable membranes to undergo this transition and to participate in membrane fusion (Hazel, 1995).

The physical and chemical characteristics of biological membranes are probably predicated by both physiological function and the specific microenvironment to which they are exposed. Similarly, adjustments in membrane physical and chemical properties, made in the face of varying thermal environments, must be within the constraints imposed by functional requirements and the need for compatibility with the membrane microenvironment. We have reported how two discrete membrane domains, the basolateral and the brush-border membranes, respond to acclimation temperature in opposite fashions. We suggest that bile, a constituent of the BBM microenvironment, may impose unusual physical requirements (inverse compensation) for this membrane domain. The physical properties observed in BBMs appear to be modulated by changes in cholesterol content with acclimation temperature.

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