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Structural order of membranes and composition of phospholipids in fish brain cells during thermal acclimatization

(temperature adaptation/membrane fluidity/fatty acids)

CSABA BUDA*, INDRANIL DEY*, NANDOR BALOGH†, LASZLO I. HORVATH†, KATALIN MADERSPACH*, MIKLOS JUHASZ‡, YOUNG K. YEO*§, AND TIBOR FARKAS*¶

*Institute of Biochemistry and †Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; and ‡Institute of Biochemistry, József Attila University, Szeged, Hungary

Contributed by Tibor Farkas, May 6, 1994

ABSTRACT **A comparison of the structural orders of membranes of a mixed brain-cell population isolated from *Cyprinus carpio* L. acclimated to either summer (23–25°C) or winter (5°C) revealed a high degree of compensation (80%) for temperature, as assayed by electron spin resonance spectroscopy. The cells rapidly forget their thermal history and adjust the physical properties of the membranes when shifted to the other extreme of temperature either *in vivo* or *in vitro*. Phospholipids separated from both types of animals exhibit only around 10% compensation. Arachidonic and docosahexaenoic acids are the major polyunsaturated fatty acids in the brains, but the fatty acid composition of the brain total phospholipids does not vary with adaptation to temperature. Separation of phosphatidylcholines and phosphatidylethanolamines into molecular species revealed a 2- to 3-fold accumulation of 18:1/22:6, 18:1/20:4, and 18:1/18:1 species in the latter; 18:0/22:6 showed an opposite tendency. Molecular species composition of phosphatidylcholines did not vary with the temperature. The same trends of changes were seen with brains of freshwater fish from subtropical (*Catla catla* L.) or boreal (*Acerina cernua*) regions. It is concluded that the gross amount of docosahexaenoic acid (22:6) plays only a minor role in adjusting the membrane physical properties to temperature. Factors other than lipids might be involved in the adaptation processes. Due to their specific molecular architecture, molecules such as 18:1/22:6, 18:1/20:4, or 18:1/18:1 phosphatidylethanolamine might prevent the contraction of membranes in the cold and may provide an environment for some other components involved in the temperature regulation of physical properties of nerve cell membranes.**

Most poikilotherms respond to thermal changes by adapting the physical properties of their membranes to the new situation to preserve the functional and structural integrity of these structures, a phenomenon that Sinensky (1) termed "homeoviscous adaptation." The homeoviscous efficacy, the extent to which the cells compensate for temperature changes, varies among the tissues and membranes (2, 3). Adjustment of the physicochemical properties of the membranes to the temperature is expected to be rapid and reversible to ensure proper functioning under fluctuating thermal conditions in fish. Wodtke and Cossins (4) have shown that the fluidity of the mitochondria in fish liver follows changes in the environmental temperature. It has also been demonstrated that the plasma membrane of carp erythrocyte rapidly adjusts to temperature under both *in vivo* and *in vitro* conditions (5, 6). The functions of neural tissue are highly dependent on membrane processes. Adaptation of the physical state of the synaptic vesicles in fish brain (2, 7), of the synaptic vesicles, mitochondria, and myelin fractions of an air-breathing, subtropical fish, *Channa punctatus* (8), and of the synaptosomal and myelin fraction of carp brain (9) has been described. Changes in environmental temperature have been shown to cause an alteration in conduction in axons of goldfish (10) and in the velocity of conduction in the vagus nerve of carp (9). All the above experiments were carried out with fish exposed to the experimental temperature for a prolonged time, and since adaptation of the membrane physical state to temperature takes place quite rapidly in fish erythrocytes (5, 6) and the liver endoplasmic reticulum (4), it seemed interesting to test whether this response was also present in the brain cells. The freshwater fish *Cyprinus carpio* was selected for this study because of its eurythermic nature and the abundance of data concerning its lipid composition and metabolism. For comparison, brains of freshwater fish evolutionarily adapted to the two temperature extremes were also investigated.

MATERIALS AND METHODS

Experimental Design. Carp (*Cyprinus carpio* L.) of 1.0–1.5 kg were obtained from a local fish farm. They were maintained in well-aerated, recirculated, and thermostatted aquaria at 25°C or 5°C. Warm temperature-acclimated ("warm"-acclimated) fish were collected in the summers of 1992 and 1993 at water temperature of 25°C. Cold-acclimated fish were collected in the winter of 1992–1993 at water temperature of 5°C. Brains were collected also from *Acerina cernua* captured in Vaasa, Finland, at a water temperature of 5°C and from *Catla catla* captured in West Bengal, India, at a water temperature of 25°C.

For warm-acclimated carp, the temperature was shifted down from 25°C to 5°C in steps of −0.5°C/hr, whereas for cold-acclimated carp, the temperature was shifted up from 5°C to 25°C with 0.5°C/hr steps. In the *in vitro* experiments, brains of both warm- and cold-acclimated fish were used. In every case, either *in vivo* or *in vitro*, at least five fish were involved.

Preparation and Incubation of Isolated Brain Cell Suspensions. Isolated brain cell suspensions were prepared by a sieving method (11). After dissection of the entire brain, the meninges were removed and the brains were finely chopped in Hanks' balanced salts solution (Ca^{2+}- and Mg^{2+}-free) (HBSS) at 25°C for warm-acclimated and temperature downshifted fish, and at 5°C for cold-acclimated and tempera-

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Abbreviations: DPH-PA, 3-[*p*-(6-phenyl-1,3,5-hexatrienyl)phenyl]-propionic acid; 14-PGSL, 1-palmitoyl-2-[14-(4,4-dimethyl-*N*-oxyl)-stearoyl]-*sn*-glycero-3-phosphoglycerol.
§Visiting scientist from Kyung Pook National University, Taegu, Korea.

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ture-up shifted fish. Cell viability was shown by trypan blue exclusion to be 95%.

Brain cell suspensions obtained from warm- and cold-acclimated fish were incubated at the alternative temperature for 80 min, subsequently restored to their acclimatization temperature, and then kept at that temperature for another 80 min. During this time, fluorescence anisotropy measurements were carried out on 100-μl aliquots of cell suspension at intervals of 10 min throughout the duration of incubation.

Steady-State Fluorescence Anisotropy Measurements. Cells were exposed to the opposite (experimental) temperature extremes for various times and then were labeled with 5 μl of 1 mM fluorescent dye 3-[*p*-(6-phenyl-1,3,5-hexatrienyl)phenyl]propionic acid (DPH-PA) (Molecular Probes) *in situ* and steady-state fluorescence anisotropy measurements were carried out at the incubation temperature by using a computer-controlled thermostatable spectrophotofluorimeter (Hitachi MPF-2A) according to Dey and Farkas (5). It has been found that DPH-PA labels the outer leaflet of plasma membrane (5, 6, 12). To correct the fluorescence intensity and anisotropy for light scattering, measurements were also made on an unlabeled sample under the same conditions as for the labeled samples. These measurements were based on Kuhry *et al.* (13). The error of these determinations was <0.005 unit.

To study the effect of temperature on membrane structural order in the spectrophotofluorimeter cell itself, a different approach was followed. Concentrated cell suspensions from warm- and cold-acclimated fish were labeled in HBSS at 25°C and 5°C, respectively. They were then kept at their respective temperature for 20 min to ensure the labeling of the cells and equilibration of the fluorescent dye in the membrane. Next, they were transferred into the precooled (5°C) or preheated (25°C) chamber of the spectrophotofluorimeter in a concentration to give 0.05 OD unit, and the changes in the anisotropy were recorded at intervals of 2 min until the anisotropy assumed a constant value.

Electron Spin Resonance (ESR) Spectroscopy. A cell suspension containing 1 mg of phospholipid was mixed with 10 μg (1 mg/ml in ethanol) of 1-palmitoyl-2-[14-(4,4-dimethyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphoglycerol (14-PGSL) in HBSS at room temperature for 15 min. Samples were contained in 1-mm (i.d.) capillary tubing. Phospholipid (5 μM) in chloroform was mixed with 15 nmol of 14-PGSL in ethanol at room temperature for 5 min. For the labeling of phospholipids, the method of Dey *et al.* (6) was followed. The samples were transferred into the capillary and accommodated in a standard quartz ESR tube. Spectra were recorded between 5°C and 25°C with a computerized ECS-106 (Bruker, Billerica, MA) ESR spectrometer equipped with air-flow temperature regulation. The rate of motion of the spin label 14-PGSL was quantitated with the effective rotational correlation time (τ_R) according to Kivelson (14). Effective order parameters were calculated by the formula of Jost *et al.* (15).

Lipid Extraction and Analysis. Total lipids were extracted from the brains or the washed cells according to Folch *et al.* (16). Phospholipids from the total lipids were separated by silicic acid column chromatography, using chloroform to remove the neutral lipids and methanol to elute the polar lipids. Phospholipid subclasses were separated by one-dimensional TLC according to Fine and Sprecher (17). Phosphorus content was determined according to Rouser *et al.* (18). Methyl esters were separated on 10% FFAP on 80- to 100-mesh Supelcoport (Supelco) in a 2-m column (2-mm i.d.). A Hitachi model 263-80 gas chromatograph connected to a Hitachi model 263-80 data processor was used. Peaks were identified by comparison with authentic standards (6).

Molecular species composition was determined according to Takamura *et al.* (19). The dinitrobenzoyl derivatives of diacylglycerols, obtained by phospholipase C hydrolysis (from *Bacillus cereus* for phosphatidylethanolamines and from *Clostridium perfringens* for phosphatidylcholines) (Sigma) of the phospholipids were separated by HPLC (Waters model 440) on a Nucleosil C_{18} column [5-μm particle size; 4 mm (i.d.) × 250 mm] using acetonitrile/2-propanol (80:20, vol/vol) of HPLC grade (Carlo Erba, Milan) isocratically as the mobile phase (flow rate, 1.0 ml/min) and monitoring the eluent at 254 nm. The peaks were recorded and calculated by using a data processor (Hitachi model 263-80). Peaks were identified by 1,2-diacylglycerol derivatives of authentic standards (6) and their relative elution times (20).

RESULTS

The carp brain total phospholipids proved to be rather rich in docosahexaenoic acid. Ethanolamine phosphoglycerides were richer in docosahexaenoic acid than choline phosphoglycerides, but the difference between cold-acclimated and warm-acclimated animals was not significant (Table 1). The ratio of saturated to unsaturated fatty acids varied with the temperature, being lower in the cold-adapted fish (Table 1). High levels of docosahexaenoic acid have been reported from

Table 1. Fatty acid composition of total phospholipids, phosphatidylcholines (PC), and phosphatidylethanolamines in carp brain in relation to acclimation temperature

	wt % (mean ± SD)					
	Total		PC		PE	
Fatty acid	5°C	25°C	5°C	25°C	5°C	25°C
16:0	13.4 ± 4.2	13.8 ± 3.2	17.1 ± 6.9	19.4 ± 7.7	8.1 ± 3.6	8.5 ± 4.2
16:1	7.9 ± 3.1	8.5 ± 1.5	7.5 ± 2.7	6.8 ± 3.0	7.6 ± 4.6	9.4 ± 0.8
18:0	7.5 ± 1.9	12.5 ± 1.3	11.2 ± 6.5	15.4 ± 12.0	5.3 ± 1.3	9.9 ± 5.5
18:1(n − 9)	20.5 ± 3.1	17.2 ± 1.4	20.7 ± 5.4	18.6 ± 8.1	16.8 ± 5.4	16.5 ± 1.0
18:2(n − 6)	1.1 ± 0.6	0.6 ± 0.5	0.8 ± 0.3	0.5 ± 0.5	1.4 ± 1.3	0.1 ± 0.1
18:3(n − 3)	2.4 ± 0.2	1.0 ± 0.6	2.5 ± 0.5	1.4 ± 0.3	2.6 ± 0.4	1.5 ± 0.1
20:4(n − 6)	10.6 ± 2.0	8.6 ± 0.6	9.7 ± 0.5	8.1 ± 6.5	12.9 ± 1.8	8.5 ± 0.8
20:5(n − 3)	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.7 ± 0.6	0.6 ± 0.6
22:4(n − 6)	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	0.6 ± 0.4
24:1(n − 9)	4.7 ± 1.2	3.3 ± 0.5	4.7 ± 2.6	3.8 ± 2.1	1.3 ± 0.5	1.2 ± 0.2
22:5(n − 3)	0.8 ± 0.3	0.3 ± 0.1	0.8 ± 0.6	0.7 ± 0.5	1.3 ± 0.5	0.4 ± 0.2
22:6(n − 3)	25.5 ± 5.2	30.5 ± 0.7	21.8 ± 5.4	18.9 ± 7.1	34.1 ± 4.0	31.7 ± 2.0
Others	4.9	3.0	2.4	5.9	7.1	11.5
(sat/unsat)*	(0.26)	(0.35)	(0.33)	(0.53)	(0.15)	(0.22)

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other fish neural elements (9, 21, 22), but, as in our case, an effect of environmental temperature on docosahexaenoic acid has not been observed. Chang and Roots (21, 22) found that a reduction in the ratio of saturated to unsaturated fatty acids in the mitochondria and microsomes accompanied cold adaptation in goldfish (*Carassius auraus*) brains, but carp nerve phospholipids (9) and garfish (*Lepisosteus osseus*) axon phospholipids (22) did not show a similar response.

There were characteristic differences with acclimation temperature in molecular species composition between phosphatidylcholines and phosphatidylethanolamines of carps. Similar differences were seen also with fish evolutionarily adapted to the temperature (Tables 2 and 3). The 18:0/22:6, 18:0/20:4, and 16:0/18:1 species were predominant in the phosphatidylcholines, with additionally 16:0/22:6 in the phosphatidylethanolamines. However, 16:0/18:1 was only a minor component in the phosphatidylethanolamine fraction. The level of 18:0/22:6 tended to be higher in the phosphatidylcholines and phosphatidylethanolamines in the brains of warm-acclimated fish than those in cold-acclimated fish. In agreement with findings in the present study, the level of 18:0/22:6 has been found to be low in the brains of marine and freshwater fish adapted to low environmental temperature (20, 23). A characteristic difference between the two phospholipids is observed in the level of 18:1/22:6, which was significantly higher in the phosphatidylethanolamines than in the phosphatidylcholines in both cold- and warm-adapted animals. Moreover, cold-acclimated fish accumulated 2–3 times more of this species, along with 18:1/20:4 and 18:1/18:1, than the warm-acclimated fish, and its level also varied slightly with an upshift of the temperature (from 14.1 ± 1.8 to 10.7 ± 0.7 wt % in the case of 18:1/22:6). Elevated levels of 18:1/22:6 phosphatidylethanolamine in the livers of freshwater and marine fish adapted to low temperature have already been reported (23–25). The rotational correlation time of 14-PGSL embedded in phospholipid vesicles prepared from brains of cold-acclimated carp was less than for warm-acclimated carp (Fig. 1), indicating a slightly less ordered environment of the spin probes in the former. The homeoviscous efficacy, calculated according to Wodtke and Cossins (4), was estimated to be around 10%. When cold- or warm-acclimated fish were shifted to the opposite extreme of the temperature in steps of 0.5°C/hr, neither the fatty acid composition (data not shown) nor the effective rotation correlation time (Fig. 1) of the isolated phospholipids was adapted to the new temperature. In contrast, vesicles from cold-acclimated temperature-upshifted animals become less ordered in the lower temperature regions and vice versa. In a separate set of experiments, the isolated brain cells were labeled with the spin probe to study the responses given by the intact membranes. To avoid thermal shock to the cells, both the preparation and the labeling procedure were done at the acclimation temperature; moreover, the ESR tempera-

Table 2. Molecular species composition of brain phosphatidylethanolamines in fish seasonally or evolutionarily adapted to contrasting temperatures

	% total			
	At 5°C		At 25°C	
Acyl groups	*C. carpio* (*n* = 8)	*A. cernua* (*n* = 5)	*C. carpio* (*n* = 6)	*C. catla* (*n* = 7)
22:6/22:6	2.6 ± 1.2	0.5 ± 0.3	2.7 ± 2.8	1.3 ± 0.7
20:5/20:5	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.7 ± 0.5
20:4/20:4	6.0 ± 1.0	2.0 ± 0.9	2.1 ± 0.7	0.8 ± 0.3
18:1/20:5	0.5 ± 0.2	2.0 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
16:1/16:1	0.3 ± 0.2	0.8 ± 0.4	0.2 ± 0.1	Trace
18:1/22:6	14.1 ± 1.8	14.4 ± 1.6	5.1 ± 2.4	2.5 ± 1.5
16:0/22:6	12.9 ± 3.9	26.1 ± 2.5	13.8 ± 2.1	27.3 ± 2.1
18:1/20:4	7.2 ± 2.5	3.4 ± 1.2	2.1 ± 1.0	2.3 ± 1.5
16:0/20:4	2.3 ± 1.5	1.6 ± 1.4	2.5 ± 0.5	1.2 ± 0.9
18:0/20:5	1.6 ± 0.5	4.0 ± 1.8	0.9 ± 0.3	0.7 ± 0.2
18:0/22:6	24.8 ± 4.4	32.4 ± 2.5	39.1 ± 8.6	49.1 ± 5.5
18:0/20:4	9.8 ± 1.0	4.8 ± 2.5	8.5 ± 2.0	4.3 ± 2.1
18:1/18:1	6.6 ± 1.4	2.1 ± 1.4	3.7 ± 1.2	0.4 ± 0.1
16:0/18:1	4.0 ± 1.7	2.2 ± 0.8	5.5 ± 0.4	3.6 ± 0.3
16:0/16:0	0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.4	0.4 ± 0.3
18:0/22:4	0.4 ± 0.2	0.1 ± 0.08	0.4 ± 0.2	Trace
18:0/18:1	2.0 ± 1.0	0.3 ± 0.1	2.7 ± 1.5	Trace
16:0/18:0	1.6 ± 1.3	1.0 ± 0.9	4.0 ± 3.3	3.8 ± 2.5
18:0/18:0	0.4 ± 0.2	0.3 ± 0.2	0.7 ± 0.4	0.3 ± 0.2
Others	8.9	1.5	4.8	0.8

Table 3. Molecular species composition of brain phosphatidylcholines in fish seasonally or evolutionarily adapted to contrasting temperatures

	% total			
	At 5°C		At 25°C	
Acyl groups	*C. carpio* (*n* = 8)	*A. cernua* (*n* = 5)	*C. carpio* (*n* = 6)	*C. catla* (*n* = 7)
22:6/22:6	1.9 ± 0.9	1.9 ± 0.8	1.7 ± 0.7	0.6 ± 0.4
20:5/20:5	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4/20:4	1.6 ± 0.4	1.8 ± 0.8	1.6 ± 0.4	0.5 ± 0.1
18:1/20:5	0.3 ± 0.2	1.2 ± 0.2	0.5 ± 0.2	0.4 ± 0.1
16:1/16:1	0.2 ± 0.1	0.8 ± 0.3	Trace	0.1 ± 0.08
18:1/22:6	3.5 ± 1.3	5.6 ± 0.6	1.3 ± 0.3	3.0 ± 1.2
16:0/22:6	11.2 ± 5.1	24.4 ± 6.7	8.4 ± 5.1	27.3 ± 10.2
18:1/20:4	1.7 ± 0.6	1.0 ± 0.5	2.6 ± 2.0	2.3 ± 2.1
16:0/20:4	3.2 ± 0.9	2.9 ± 0.7	2.6 ± 0.7	1.2 ± 0.3
18:0/20:5	1.0 ± 0.2	1.8 ± 0.5	0.4 ± 0.3	0.7 ± 0.2
18:0/22:6	12.6 ± 2.7	10.0 ± 3.1	23.5 ± 15.0	19.5 ± 3.1
18:0/18:2	2.7 ± 2.0	Trace	2.3 ± 2.0	Trace
18:0/20:4	12.7 ± 8.5	2.5 ± 1.1	13.5 ± 2.4	2.5 ± 1.5
18:1/18:1	3.4 ± 0.1	1.6 ± 0.8	2.0 ± 1.2	1.8 ± 1.4
16:0/18:1	21.5 ± 12.8	24.6 ± 14.6	20.4 ± 14.5	30.5 ± 18.7
16:0/16:0	3.4 ± 1.1	1.7 ± 1.2	2.8 ± 1.7	4.1 ± 2.1
18:0/18:1	3.5 ± 2.8	0.8 ± 0.6	3.7 ± 2.7	0.8 ± 0.5
16:0/18:0	8.5 ± 4.7	9.4 ± 3.2	8.7 ± 4.9	9.4 ± 3.3
18:0/18:0	1.4 ± 0.5	0.2 ± 0.1	1.6 ± 1.4	Trace
Others	5.2	11.3	2.2	3.0

See legend to Table 2.

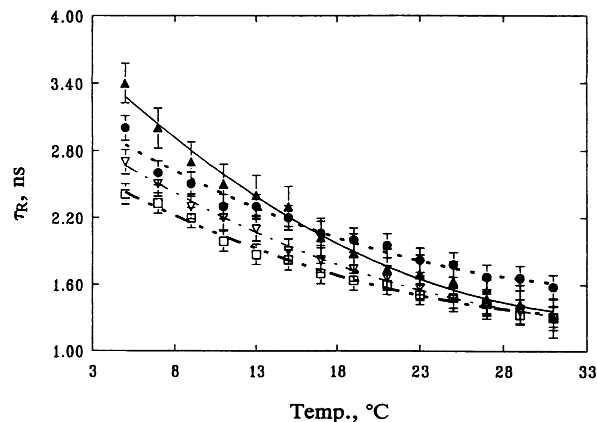

FIG. 1. Temperature dependence of the rotational correlation time (τ_R) of 14-PGSL embedded in phospholipid vesicles prepared from brains of cold (5°C) and warm (25°C) temperature-acclimated

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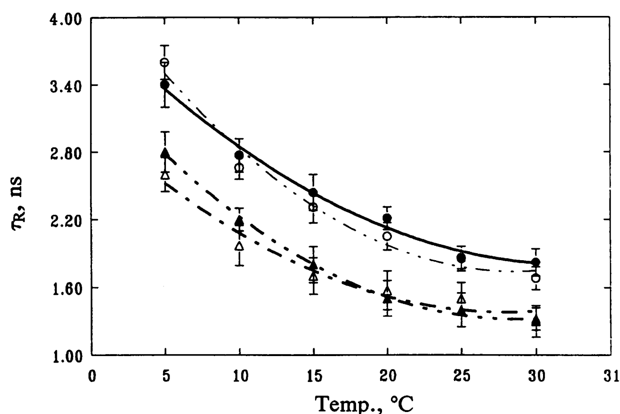

FIG. 2. Temperature dependence of the rotational correlation time (τ_R) of 14-PGSL embedded in membranes of brain cells of carps adapted to summer or winter temperature or shifted to the other temperature extreme (means ± SD of five experiments). ●, Warm-acclimated; △, shift down; ▲, cold-acclimated; ○, shift up.

ture scannings were run in the heating cycle for the cold-adapted cells, and in the cooling cycle for the warm-adapted cells. This experimental approach, in sharp contrast to the isolated phospholipids (Fig. 1), demonstrated an ≈80% compensation of the membrane structural order for the temperature (Fig. 2). There were no significant differences in the effective rotation correlation times of warm-acclimated and cold-acclimated temperature-upshifted cells and vice versa (Fig. 2).

This experiment suggested that adjustment of the membrane physical state of the brain cells to the new temperature was a rapid and reversible process. To confirm this, brain cells prepared from warm-adapted fish were cooled under *in vitro* conditions to 10°C and the time course of the fluorescence anisotropy of DPH-PA embedded in the plasma membrane was followed, after the removal of aliquots of the cells from the incubation medium. The anisotropy parameter started to decrease shortly after the cells were exposed to the reduced temperature, and it assumed a constant low value after 15–20 min (Fig. 3). When the same cell population was rewarmed, the anisotropy increased and reached the original value within a few minutes. Similar results were obtained when cells from warm- or cold-adapted fish brains were exposed to cold or warm temperature, respectively, in the cell of the spectrophotofluorimeter. In a typical experiment in which cells from cold-adapted fish were dropped into the spectrophotofluorimeter cell set to 25°C, the fluorescence anisotropy started to increase within a few minutes (Fig. 3

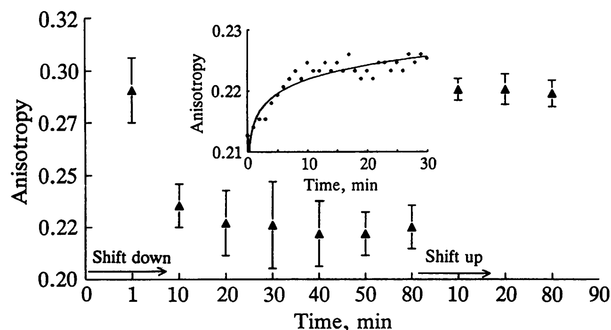

FIG. 3. Effect of temperature shift *in vitro* on fluorescence anisotropy of DPH-PA embedded in membranes of brain cells prepared from warm-adapted fish (means ± SD of five experiments). (*Inset*) Change in fluorescence anisotropy of DPH-PA in brain cell

Inset). We interpret these results as a manifestation of an active adaptation of the physical properties of the intact brain cell membrane to the new temperature. Without this adaptation, opposite results would have been obtained—i.e., a decrease in the anisotropy parameter in temperature-upshifted cells, and vice versa, as demonstrated in model experiments using phospholipid vesicles (data not shown).

DISCUSSION

Fatty acid composition in mammalian brain cell is fairly stable: only long-term dietary effects can cause some changes in it (26–28), especially in the phosphatidylethanolamine fraction (27, 29). Long-chain polyunsaturated fatty acids present in high concentration in mammalian nerve cells originate in the liver and are transported to the brain by the circulatory system (30). The same is also undoubtedly true for fish, but the effect of the environmental temperature must also be taken into consideration. Ethanolamine phosphoglycerides gave the most sensitive response to changes of the environmental temperature. Despite the expected slowing down of metabolic processes with decreasing temperature, a turnover of molecular species composition of the phosphatidylethanolamines took place in the brains of thermally acclimated carp. The changes were characterized by accumulation of 1-monounsaturated/2-polyunsaturated and dioleoyl species with a fall in the environmental temperature (Table 2). It seems conceivable that these changes are a direct effect of temperature on the lipid metabolism of carp brain and do not stem from the diet, since these fish stop feeding at or below 10°C. Previous studies demonstrated increases in the formation (31) and level (32) of long-chain polyunsaturated fatty acids in carp liver acclimated or exposed to cold, and it is tempting to speculate that a proportion of these fatty acids might have been transported to the brain during the acclimation to reduced temperatures. Fish brain cells have been shown to take up and selectively incorporate unsaturated fatty acids from the incubation medium *in vitro* (33, 34). The level of 18:1/22:6 phosphatidylethanolamine in the brain cells of carp acclimated to reduced temperatures resembles that which has been found in the brains of boreal and subtropical fish species (Table 2) as well as in brains of cod and rainbow trout adapted to low (5–7°C) temperatures (20, 23). It requires further investigations to decide whether the accumulation of this species in response to cooling is a result of a selective deacylation/reacylation reaction, an intensive desaturation of the existing 18:0/22:6 species, or its transfer from the liver. In another connection, it has been shown that carp liver cells preferentially esterify 18:1 at the *sn*-1 position of phosphatidylethanolamine (32) under *in vitro* conditions in the cold and, moreover, that high levels of this species characterize the phosphatidylethanolamines in the livers of marine and freshwater fish evolutionarily adapted to low temperatures (24).

Parallel to changes in the acyl composition of the brain phosphatidylethanolamines, there was also a high level (70–80%) of compensation of membrane fluidity for temperature changes, as measured by spin label and fluorescence polarization techniques. Although the individual membranes were not separated in this study, based on the data obtained for the brains of rat (28) and *Channa punctatus* (8) and for the nerve of carp (9), we propose that the fatty acid composition of carp brain total phospholipids is close to that of the plasma membranes. Thus, it can be speculated that both labels (14-PGSL and DPH-PA) indicate the ordering state of these structures. However, it still remains to be elucidated whether the observed changes in acyl-group composition of phosphatidylethanolamines are directly related to the observed

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dominant phosphatidylcholine species (16:0/18:1, 16:0/22:6, and 18:0/22:6) are rather close (35, 36) and the same might be true also for the phosphatidylethanolamines. Indeed, the compensation was only about 10% when total phospholipids were assayed (Fig. 1).

A principal difference between the extracted total phospholipids and the intact membranes is the presence of the proteins in the latter. One of the functional roles of 1-monounsaturated/2-polyunsaturated phosphatidylethanolamines might be the prevention of the contraction of the bilayer due to reduced thermal motion and thereby the maintenance of a higher degree of disorder in cold. We speculate that certain nonlipid membrane components, such as membrane proteins, are responsible for the observed high degree of compensation of membrane ordering state for the temperature. Due to their specific molecular architecture and their effects on packing properties of the bilayer these phosphatidylethanolamines might contribute to these proteins assuming the appropriate configuration in cold. Phosphatidylcholines such as 16:0/18:1 or 16:0/22:6 may not share this property of phosphatidylethanolamines and serve merely as a matrix of the membranes.

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