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Pressure acclimation of the eel and liver membrane composition

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Abstract. Homeoviscous adaptation of membrane fluidity is known to exist in fish living at great depths. Is this adaptation also present in fish living near the surface but experimentally acclimated to high pressure? The composition of mitochondria-rich fractions extracted from the livers of eels acclimated for 15 days at 101 ATA was determined. The results show that pressure induced a significant increase (+100%) of total phospholipids (PL) and cholesterol without a change in their ratio. The increase of PL content was accompanied by a decrease in phosphatidylcholine in favour of phosphatidyl ethanolamine which, due to its preference for the H_{II} form, is able to compensate for the loss in fluidity induced by pressure.
Key words. Eel; hydrostatic pressure; liver; mitochondria; phospholipids.

Homeoviscous adaptation of membrane fluidity is well documented as a response to changes in environmental temperature[1,2]; briefly, an increase in temperature is associated with an increase in membrane fluidity. The thermodynamic relationships involving temperature and pressure lead us to suppose that a decrease in temperature can resemble the effects of high pressure (see ref. 3 for review). This hypothesis has been tested for homeoviscous adaptation, it has been found that homeoviscous adaptation exists in bacteria[4,5], and also in deep water fish species. When liver cell membrane fluidity is measured at 1 ATA, it is higher in fish living at a considerable depth than in congeneric fish living near the surface[2]. Knowing that the yellow freshwater eel (a shallow-water species) is able to adapt its energy metabolism to high pressure[6,7], it was interesting to ask whether there are also changes in liver membrane composition, as shown in deep-water species. The composition of the mitochondria-rich fraction (MRF) isolated from fresh liver was therefore determined in eels exposed to 101 ATA hydrostatic pressure (1000 m depth) for 15 days, and compared to that in control fish kept at 1 ATA.

Material and Methods

Animals. 12 yellow freshwater eels (*Anguilla anguilla* L.) were used. They averaged 80 g in weight and were obtained from a regional fishery. Fish were stored without feeding in polyethylene tanks filled with tap-water which was continuously renewed and aerated. The temperature was kept constant at 17 °C.

Protocol. Six eels were introduced into the experimental tank (volume 17.8 l), which was placed in a hyperbaric chamber (130 l) and connected to a high-pressure water circulation system[8]. Water oxygen content and temperature were continuously monitored using a probe placed in the experimental tank. Eight days later, the hyperbaric chamber was air-compressed at a rate of 2 atm.min^{-1} up to 101 ATA. This pressure was maintained for 15 d, then decompression was performed at a rate of 2 atm.min^{-1} until 51 ATA was reached then 0.2 atm.min^{-1}. During this experiment, 6 fish were kept at 1 ATA under the same experimental conditions, as a control group. At the end of the decompression period, fish were sacrificed by decapitation and samples of liver removed.

Sample preparation. Liver samples were chopped with scissors, then homogenized on ice with a polytron homogenizer (2 passes of 5 sec, then 10 passes of a glass-teflon Potter homogenizer), in a medium containing 320 mM sucrose, 10 mM imidazole-HCl (pH 7.8 at room temperature), 1 mM EDTA and 1% bovine serum albumin: 9 ml of solution was used per gram of tissue. The suspension was centrifuged twice at 1,000 *g* for 10 min (4 °C). The supernatant was removed with a Pasteur pipette and spun at 10,000 *g* for 10 min (4 °C). The tan pellet containing mitochondrial membranes was resuspended in a small volume (0.3 ml) of isolation medium and immediately stored at −80 °C: this suspension was termed MRF (mitochondria rich fraction).

Lipid analysis. Phospholipid (PL) content was measured using the Bartlett method[9], while an enzymatic method[10] was used to measure cholesterol (CH).

The results for CH and PL are expressed relative to protein (PT) level, which was determined according to the method of Lowry et al.[11]. To measure the different PL subfractions, a monodimensional separation was performed by HPTLC[12]. The PL subfraction levels were subsequently measured using densitometry (Cliniscan densitometer, Helena).

Data analysis. Statistical significance of the results was evaluated at the 5% level with Student's t-test.

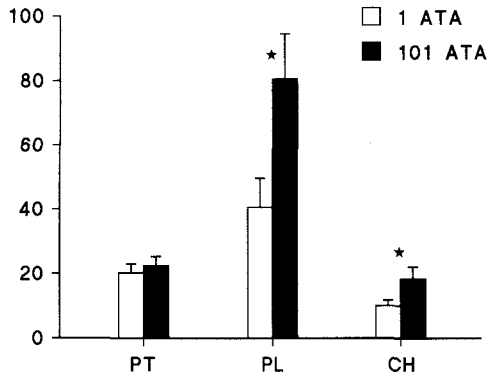

Figure 1. Levels of proteins (PT), phospholipids (PL) and cholesterol (CH) in membrane extracts from liver of eels at 1 ATA or acclimated at 101 ATA hydrostatic pressure for 15 days.
PT is expressed in $mg.g_{ww}^{-1}$; PL, CH are expressed in nmol. mg_{PT}^{-1}.
*Significant difference at the 5% level or better.

Table. % composition of phospholipids in mitochondria rich fractions extracted from liver of eels at 1 ATA and after 15 days at 101 ATA hydrostatic pressure.

	1 ATA	101 ATA	P
SM	9.6 ± 0.2	9.8 ± 0.4	NS
PC	59.2 ± 2.7	50.5 ± 1.2	0.05
PS	3.9 ± 0.8	3.1 ± 0.6	NS
PI	7.7 ± 0.5	7.7 ± 1.2	NS
PA	7.9 ± 0.8	9.3 ± 0.4	NS
PE	11.6 ± 1.4	19.8 ± 1.5	0.01

SM: sphyngomyelin; PC: phosphatidylcholine; PS: phosphatidylserine; PI: phosphatidylinositol; PA: phosphatidic acid; PE: phosphatidylethanolamine.

Results

The changes in the concentrations of proteins (PT), phospholipids (PL) and cholesterol (CH) are illustrated in figure 1. Acclimation to high pressure significantly increased both PL and CH ($p < 0.05$) by about 100%: consequently the CH/PL ratio was not modified (0.27 ± 0.04 at 1 ATA, 0.23 ± 0.02 at 101 ATA). The absolute tissue content of proteins was not modified, but the level is greatly reduced relative to that of PL. When the percentage composition of the PL was examined (table), the only significant change was an increase of phosphatidylethanolamine (PE; $p < 0.01$) at the expense of phosphatidylcholine (PC; $p < 0.05$) in the 10% range in acclimated eels.

Discussion

Fish are very interesting models in physiology and ecophysiology because they are present in numerous types of environment. The environments can differ in oxygenation, light, food availability, salinity, temperature and pressure. Adaptation to different environments means that congeneric species can exhibit very different biochemical and physiological features. An example of remarkable adaptation to environment is the so called homeoviscous adaptation, which consists of a modulation of membrane fluidity in response to changes in temperature and/or pressure [1–4,13].

In the study described here, changes in liver MRF were explored in yellow freshwater eels acclimated for 15 days to 101 ATA hydrostatic pressure. The first interesting result is the absence of absolute changes in PT content, whereas PL and CH levels increased by about 100%, so that the relative amount of PT decreased (by about 50%) which is in agreement with several previous reports (see ref. 14 for review). The question is why such an increase in PL and CH should occur. One explanation is that the increase in PL content in the MRF is evidence for liver activity providing other tissues with PL, and also with FFA more suitable for ensuring optimum membrane fluidity. Moreover what is observed in MRF also probably reflects changes at the membrane level. Such a hypothesis is functionally important because it could mean that proteins embedded in membranes (enzymes or enzyme complexes, with or without cofactors, ion channels etc.) are relatively less abundant and consequently have fewer possibilities for interaction: this must be compensated for by an increase in the efficiency of enzymatic reactions, as previously suggested[14].

The second interesting result from this work concerns the significant and concomitant increase (+100%) of PL and CH without any change in the CH/PL ratio, which is very low compared to that in other fish tissues (see ref. 15). It is known that low temperature decreases membrane fluidity but this decrease is reduced when cholesterol is present. Considering the low temperature/high pressure thermodynamic correspondence, it can be supposed that the membrane fluidity is not altered in the liver, at least by the change in PL and CH.

In fact, total PL is doubled but the percentage composition is not strongly altered (table). The only significant change is a redistribution of PC and PE (decrease of the first, increase of the second). Although it affects only 10% of the total PL, this redistribution is probably very important from a functional point of view. It may result in a perturbation in bilayer organisation because of a new balance between $L\alpha$ (PC) and H_{II} (PE) phospholipid forms, with consequences for ion permeability and enzyme functions (see ref. 16 for review). These relative changes in PC and PE could be explained in the following manner. As temperature decreases (or pressure increases as in the present study), and cholesterol increases (as observed), this favors the conversion of phospholipids from the H_{II} form to the $L\alpha$ form, which is less fluid. This loss in fluidity can be compensated, at least partly, by the presence of a higher proportion of PL, which mainly adopts a H_{II} (PE) form, in contrast to PC which more easily adopts the $L\alpha$ form. Such a mechanism has been observed in living organisms (see

ref. 16) when growth temperature was lowered, and it allows the critical balance between bilayer and non-bilayer organisation of the membrane phospholipids to be maintained. It can be noted that the PE/PC ratio changes from 0.2 at 1 ATA to 0.4 under pressure, i.e. it is doubled, like the level of total phospholipids or cholesterol (fig. 1). The modifications in membrane composition and organisation described above can be observed without any significant change in fluidity being measured (see ref. 15). It is known that fluidity, when estimated from anisotropy measurements, is not a good indicator of the properties of membranes from liver[13].

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