Modification of the Amino Acid Pool in the Parietal Muscle of Two Euryhaline Teleosts During Osmotic Adjustment

Euryhaline fishes are classically considered as homeosmotic animals. However, changes of 20 to 30% in plasma osmotic pressure have been observed in various euryhaline teleosts after transfer from sea water to fresh water [1]. In these species, the tissues have therefore to cope with the changes in the blood osmotic pressure occurring when the animal is withstanding an osmotic stress. The participation of the amino acid pool in the cellular osmoregulation process is a well known phenomenon in euryhaline invertebrates[2,3]. In vertebrates, this aspect of cellular volume regulation has received only scant attention until now[4-6] except in two recent investigations on the hagfish[7] and on the eel[8].

We have therefore undertaken a study of the modification in ionic and amino-acid concentration occurring in the parietal muscle of 2 euryhaline teleosts when adapted to normal sea water (salinity 33‰), hypertonic or hypotonic media (salinity 70‰ and 1‰). Thick-lipped mullets, *Crenimugil labrosus* (Risso) obtained from the French Atlantic coast and southern Flounders, *Paralichthys lethostigma*, Jordan and Gilbert, collected in the vicinity of Beaufort, North Carolina, were used for these experiments. Blood was rapidly withdrawn by cardiac puncture and centifuged. Aliquots of plasma as well as samples of parietal muscle were prepared for osmotic pressure, water content, ionic and free amino acids determinations following techniques previously described[9,10].

As shown in Table II, the plasma osmotic pressure decreases of about 38% in both species when adapted from 200% sea water to fresh water. It can therefore be assumed that during the transfer of such species in these media, the tissues have to cope with a change in osmotic pressure. As a matter of fact an active regulation of the cellular volume appears to be at play in both species since there is no significant change in the muscle water content during the adaptation to media of various salinities. Free amino acids together with ions are known to be important osmotic effectors in invertebrate cells, in which they are also involved in the cellular osmotic regulation[2,3]. In the two fishes taken in their normal medium (sea water) inorganic ions account for 66% and 77% of the total osmotic pressure while amino acids represent 15.3% of the cellular osmotic pressure in the mullet and 19% in the flounder. When taken together amino acids

Table I. Changes in amino-acid level in the parietal muscle of 2 marine teleosts during their osmotic adjustment

Amino acid	*C. labrosus*			*P. lethostigma*		
	200% SW	SW	FW	200% SW	SW	FW
Lysine	0.81	0.98	1.08	1.18	1.12	1.01
Histidine	0.64	0.79	0.66	0.21	0.56	0.22
Ornithine	0.12	0.29	0.21	0.13	0.14	0.10
Arginine	0.08	0.26	0.14	0.09	0.38	0.26
Taurine	36.75	23.29	6.38	41.30	28.37	16.47
Aspartic acid	1.31	1,23	0.11	1.56	1.66	0.16
Threonine	0.86	0.38	0.35	0.50	0.64	0.41
Serine	2.10	1.30	0.45	2.27	1.91	0.53
Glutamic acid	1.37	1.12	0.38	1.44	1.80	0.40
Proline	1.18	0.81	0.29	1.46	1.42	0.66
Glycine	27.02	11.56	2.58	12.81	8.03	1.35
Alanine	8.49	3.61	0.82	5.10	4.14	0.93
Valine	0.32	0.27	0.25	0.37	0.38	0.31
Methionine	0.12	0.14	0.13	0.33	0.32	0.27
Isoleucine	0.27	0.21	0.24	0.24	0.27	0.19
Leucine	0.51	0.41	0.38	0.33	0.24	0.26
Tyrosine	0.19	0.20	0.09	0.36	0.38	0.16
Phenylalanine	0.15	0.13	0.16	0.21	0.23	0.14

Concentrations are given in m*M*/kg tissue water and are mean values of 7 to 10 animals.

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Table II. Osmotic balance and water content of *Crenimugil labrosus* and *Paralichthy lethostigma* muscles

	C. labrosus			*P. lethostigma*		
	200% SW	SW	FW	200% SW	SW	FW
Total amino acid concentration (m*M*/KgH_2O)	82.29	46.98	14.70	69.89	51.99	23.83
Na+K+Cl concentration in muscle (mEq/KgH_2O)	270.9	256.0	225.9	277.7	267.6	228.2
Osmotic pressure due to amino acids (mOsm/KgH_2O)	104.5	59.7	18.7	88.8	66.0	30.3
Osmotic pressure due to Na+K+Cl in muscle	270.9	256.0	225.9	277.7	267.6	228.2
Osmotic pressure due to the measured components (mOsm/KgH_2O)	375.4	315.7	243.6	366.5	333.6	258.5
Plasma osmotic pressure (mOsm/l)	440 ± 5	389 ± 9	270 ± 7	406 ± 4	348 ± 4	251 ± 6
Osmotic pressure due to amino acids (%)	23.75	15.35	6.92	21.87	18.97	12.07
Osmotic pressure due to ions (%)	61.57	65.81	83.66	68.40	76.89	90.92
Water content (% wet wt.)	76.61 ± 1.6	76.82 ± 1.7	79.90 ± 2.2	76.33 ± 1.5	78.74 ± 1.4	80.65 ± 1.8

The osmotic pressure of amino acid pool have been calculated asssuming that the Δ of a molar solution of amino acids is —1.86 °C[14].

and ions account for 80 to 90% of the cellular osmotic pressure (Table II). The deficit in osmotic pressure is probably made up by various organic molecules as carnosine or anserine which are known to be present in large amounts in the muscle of various fishes[4,7,11,13]. During hyper or hyposmotic stress, there is no significant variation of the muscle total ionic content. On the other hand the amino-acid concentration changes with the osmotic adaptation. When going from the hypertonic medium (200% sea water) to the hypotonic one (fresh water), there is a 82% decrease in the amino-acid content of the mullet muscle and a 66% decrease in that of the flounder muscle (Table II). These changes affect most of the amino acids and cannot be attributed to a simple dilution process since the cellular water content does not change significantly during the adaptation to the various media. Moreover, the percentage of decrease observed in the amino-acid concentration varies with each amino acid. The most important changes are observed at the level of the so-called non essential amino acids (GLY, ALA, ASP, GLU, SER, PRO)[13] and is specially important at the level of glycine, alanine and the amine taurine (Table I). Such a feature is also observed in all the euryhaline invertebrate species studied so far [2,3] as well as in vertebrate species ([4,7], this paper) and suggests the possibility that the role played by the amino acids in the intracellular isomotic regulation is a generalized mechanism existing in both invertebrates and vertebrates[15,16].

Résumé. Au cours de l'adaptation de deux poissons euryhalins à des milieux hyper- ou hypotoniques, la teneur en eau du tissus musculaire ne varie pas en dépit des modifications de la pression osmotique du plasma sanguin. Les acides aminés et particulièrement la glycine, l'alanine et une amine la taurine, paraissent impliqués dans ce processus de régulation de la pression osmotique intracellulaire.

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Numerical Hyperplasia in Human Heart Hypertrophy

It is still unknown whether in hypertrophied human hearts there is an increase in the number of heart muscle cell, i.e. heart muscle cell nuclei, or whether the enlargement of the heart muscle is related only to the enlargement of the single heart muscle fibres.

In earlier investigations (SANDRITTER and SCOMAZZONI[1] KOMPMANN et al.[2], FISCHER et al.[3]) a process of polyploidization in hypertrophic human hearts with increasing heart weight, meaning a multiplication of the DNA content per single heart muscle cell nucleus, was demonstrated by cytophotometric measurements. This finding suggested that biochemical DNA determination alone do not reflect the cell number. We therefore combined cytophotometric Feulgen DNA measurements with biochemical DNA determinations in order to calculate the number of heart muscle cells. The relative number of connective tissue cell nuclei was likewise considered.

Human hearts were obtained from autopsy material less than 24 h after death and weighed, after removal of epicardial fat and connective tissue. Samples of heart muscle, each weighing 10 g, were taken from 6 different sites of both ventricles. We have proved that these samples were representative for the whole individual heart muscle, because DNA determinations on 38 different sites of some hearts did not show any statistically significant differences in DNA amount or polyploidization pattern as measured from the 6 chosen sites. After extraction with perchloric acid, the total amount of DNA was determined by the diphenylamine reaction according to DISCHE[4] and BURTON[5]. The cytophotometric DNA measurements were performed with a Deeley Integrating Microdensitometer (DEELEY[6]) on 300 muscle nuclei per heart.

The number of connective tissue cell nuclei was determined in histological slides from 38 areas of each heart using a light microscope with a magnification of 400 ×. We calculated the number of connective tissue cell nuclei in relation to 100 heart muscle cell nuclei. Cytophotometric measurements showed that connective tissue cell nuclei all have, without exception, a diploid DNA pattern. Hydroxyproline determinations, according to STEGEMANN's method[7] was taken as a measure of the amount of the connective tissue.

The evaluation of these parameters allows the calculation of the number of heart muscle cell nuclei, taking into account the amount of total DNA of the individual heart muscle, the amount of DNA of the heart muscle cell nuclei and the number of connective tissue cells.

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