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Effect of cold exposure on polyamine levels and ornithine decarboxylase activity of goldfish tissues

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Abstract. Goldfish exposed to the cold (5 °C) for a week exhibited modifications in polyamines metabolism, as shown by the increase of putrescine levels in liver, heart, muscle and brain, whereas the content of spermidine and spermine was unchanged. Putrescine increases in tissues considered were concomitant with a remarkable rise in ornithine decarboxylase activity. Kinetic analysis of brain enzyme activity showed that the apparent K_m was unchanged by cold exposure, whereas V_{max} was strongly increased.

Key words. Goldfish; temperature; polyamines; ornithine decarboxylase.

The polyamines putrescine, spermidine and spermine represent a group of naturally occurring compounds that exert a bewildering array of biological effects; yet, despite several decades of intensive research, their exact physiological function remains obscure. Chemically these compounds are organic aliphatic cations with two (putrescine), three (spermidine) or four (spermine) amino groups that are fully protonated at physiological pH values. Polyamines are found in all living cells, and they are associated with growth and protein synthesis [1, 2]. They are also possible candidates for intracellular signal transduction, and they have been implicated in the modulation of excitatory amino acid receptors [3]. Depletion of cellular polyamine levels has been shown to lead to decreased cell growth and alterations in cell differentiation [4, 5]. On the other hand, excessive levels of polyamines may have toxic effects [6, 7]. Putrescine is formed from ornithine via decarboxylation catalysed by ornithine decarboxylase (ODC), the initial reaction in the biosynthesis of the polyamines spermidine and spermine [8, 9]. Several studies in prokaryotes and eukaryotes have shown that ODC activity and polyamine levels are highest during cell replication and differentiation, and they decrease as these processes cease. However, there is general agreement that these polycations can be greatly elevated following the application of a variety of stimuli [10].

While polyamine distribution and ODC activity have been studied extensively in mammals, relatively little work has been done concerning lower vertebrates. Polyamine distribution and metabolism have been studied in tissues from different fish [11, 12], and modifications of polyamine levels or ODC activity in different teleost tissues have recently been reported during embryonic development [13] or in response to variations in diet, salinity and heavy metal [14, 15].

Although field studies have found a correlation between water temperature and the in situ seasonal ODC activity [16, 17], the results are often conflicting, and it is unclear whether this correlation is a functional relationship or merely reflects the association between water temperature and polyamine responses. To evaluate more clearly the effect of temperature exposition on polyamine metabolism in fish tissues, ODC activity and polyamine concentration should be monitored under laboratory conditions. The aim of this work was to study the effect of short-term exposition to cold both on polyamine metabolism in various tissues from the eurthermal teleost *Carassius auratus* (goldfish), and on kinetic properties of ODC. In addition, the influence of short-term starvation was considered.

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Materials and methods

Chemicals. L-[1-^{14}C] ornithine was supplied by Du Pont NEN (Boston). All other chemicals were from Sigma Chemical Co. (St. Louis, MO, USA). α-Difluoromethylornithine (DFMO) was a generous gift from Marion Merrel Dow, Strasbourg, France.

Animals. Freshwater goldfish *C. auratus* (10–12 cm in length; mass ~35 g), obtained from a local breeder, were maintained at 20 °C in thermostated-glass aquaria filled with unchlorinated well water constantly aerated and filtered through activated charcoal filters. All fish were fed once daily with a commercial fish pellet and kept under a 12 h:12 h light:dark photoperiod. After 10 days, a group of fish was transferred to an aquarium located in a cold room, in which the temperature reached 5 °C within 9–10 h, and was maintained for 1 week before being used in experiments. A separate group of fish was maintained for 1 week at 20 °C, without food. Animals were killed by decapitation, and brain, liver, heart and white muscle (epiaxial) were quickly removed. Brains were divided into halves; all tissues were held at −80 °C in a deep freezer until analysis.

The experiments reported here were performed according to the Italian law on protection of laboratory animals, with the approval of a local bioethics committee and under the supervision of a veterinary commission for control of animal care and comfort.

Measurement of polyamine concentration. For polyamine assay, half the brain and portions of the liver, heart and white muscle were sonicated in 0.1 N perchloric acid and centrifuged for 15 min at 15,000*g*. The pellets were dissolved in 0.5 N NaOH containing 0.2% sodium dodecyl sulfate (SDS) for protein assay, while aliquots of the supernatant were subjected to fluorimetric detection after *O*-phthaldialdehyde derivatization and high-pressure liquid chromatography (HPLC) separation, as described previously [18].

Assay of ODC activity. For ODC assay, the other half of the brain, the liver, the heart and the muscle were homogenized in ice-cold 50 mM Tris-HC (pH 7.5) containing 0.1 mM EDTA, 5 mM dithiothreitol, and 0.04 mM pyridoxal-5-phosphate. After centrifugation at 20,000*g* for 20 min, aliquots of the supernatant were assayed, at 22 °C, essentially according to a previously described procedure [19], by measuring the $^{14}CO_2$ released from [^{14}C]ornithine (specific activity 40–60 mCi/mmol, final concentration 0.2 mM) and trapped by hyamine hydroxide. Blanks containing the ODC competitive inhibitor DFMO (2 mM) were run in paral-

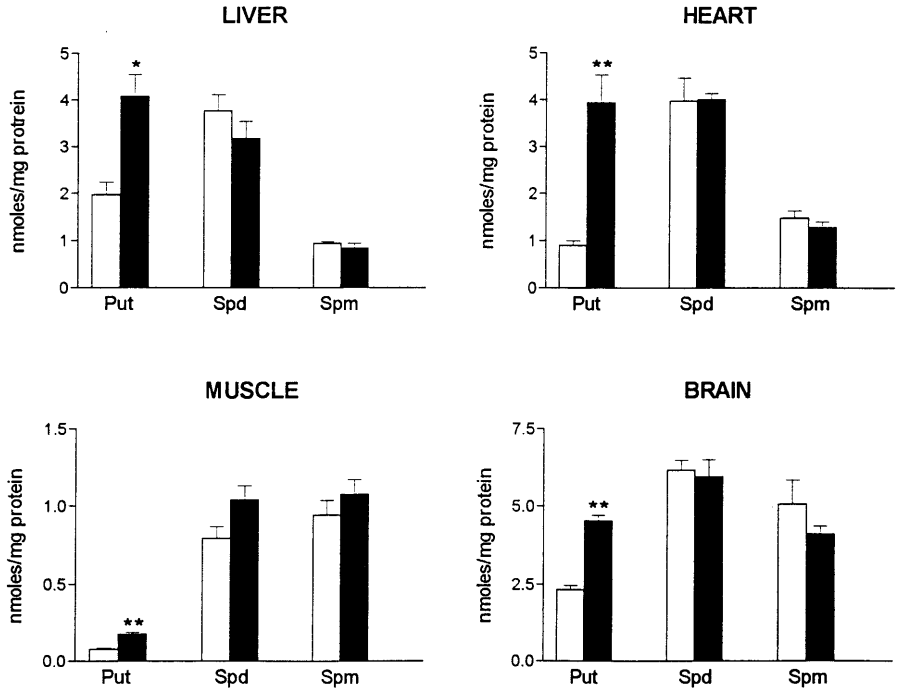

Figure 1. Concentration of putrescine (Put), spermidine (Spd) and spermine (Spm) in several tissues from adult goldfish exposed for 1 week at 20 (□) or 5 °C (■). Experimental conditions were as reported in the 'Materials and methods' section. Each bar represents the means ± SEM of values obtained from 7–11 fish. $^{**}P < 0.0001$, $^{*}P < 0.002$.

lel. The final results were expressed as nanomoles of CO_2 evolved per hour and gram of supernatant proteins [20].

Statistical analysis. Student's t-test was used to measure the significance of differences among mean values of the variables measured. The apparent K_m and V_{max} values, presented in the results, were estimated using Lineweaver–Burk double reciprocal plots (computer program Inplot 4; Graph Pad Software).

Results

Compared with 20 °C, the exposure of fish at 5 °C for 1 week caused a significant increase in putrescine levels in liver, heart, white muscle (epiaxial) and brain (107, 342, 131 and 94%, respectively), whereas no statistically significant effect on spermidine and spermine contents was observed (fig. 1). As shown in figure 2, cold exposure also strongly increased the activity of ODC. The highest increase of enzyme activity was found in the heart, whereas the smallest increase was observed in the liver. As fish exposed to cold showed hypomotility and inappetence, we assessed the effect of starvation on polyamine metabolism. The polyamine concentration in tissues of fish maintained for 1 week at 20 °C without food was not statistically different from fish fed daily (table 1). Short-term starvation also had no effect on ODC activity (data not shown).

In order to elucidate enzyme-increased activity, we analysed the kinetic parameters of ODC activity in the brain of fish exposed at different temperatures. The results of incubating brain homogenates with increasing concentrations of [^{14}C]ornithine are summarized in a kinetic saturation plot and in a Line-weaver–Burk plot (fig. 3). From these plots the apparent K_m and V_{max} values can be deduced. The apparent K_m in the brain of goldfish exposed at 20 °C was 281.3 ± 29.4 μM, and the V_{max} was 49.8 ± 2.6 nmol/g protein/hr. Enzyme activity in the brain of fish cold-exposed exhibited a similar K_m (262.9 ± 31.7 μM) to that measured in the brain of fish maintained at 20 °C, whereas V_{max} was considerably higher (360.0 ± 25.3 nmol/g protein/hr).

Discussion

The evidence presented in this paper indicates that the exposure of adult goldfish to low temperature can profoundly affect polyamine composition in different tissues. Compared with 20 °C, exposure at 5 °C for 1 week

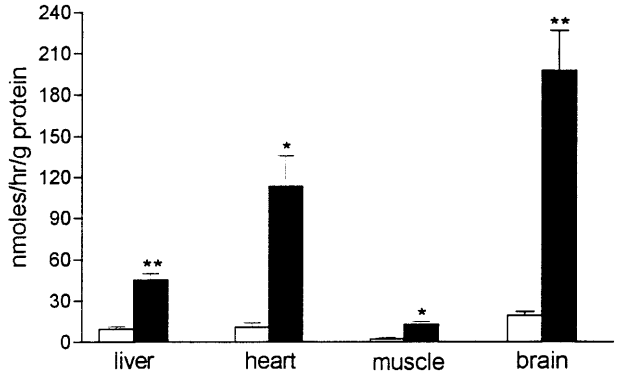

Figure 2. Ornithine decarboxylase activity in several tissues from adult goldfish exposed for 1 week at 20 (□) or 5 °C (■). Experimental conditions were as reported in the 'Materials and methods' section. Each bar represents the means ± SEM of values obtained from 7–11 fish. $^{**}P < 0.0001$, $^{*}P < 0.001$.

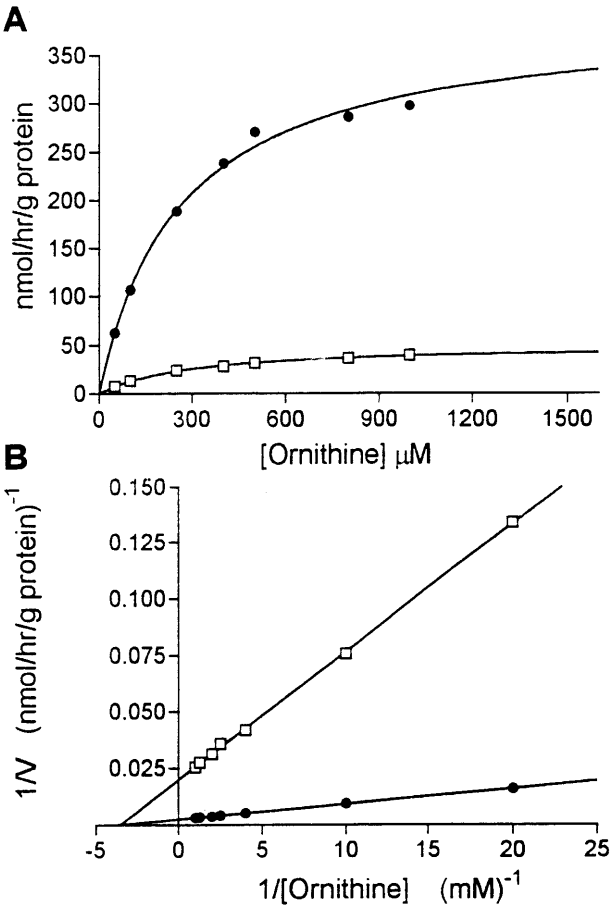

Figure 3. Kinetic analysis of ODC activity in brain homogenates from goldfish exposed for 1 week at 20 (□) or 5 °C (●). (*A*) Kinetic saturation plot; (*B*) Lineweaver–Burk plot. Each point is the mean ± SEM of triplicate determination for each dose in six separate experiments performed as described in the text. Activity was determined under standard conditions at 22 °C.

Table 1. Polyamine concentration in several tissues from adult goldfish maintained for 1 week at 20 °C without food.

		Liver	Heart	Muscle	Brain
Putrescine	control	2.13 ± 0.55	1.21 ± 0.47	0.08 ± 0.02	2.58 ± 0.47
(nmol/mg protein)	no food	2.80 ± 0.81	1.13 ± 0.58	0.12 ± 0.07	1.72 ± 0.36
Spermidine	control	3.95 ± 0.54	4.30 ± 0.81	0.72 ± 0.25	5.99 ± 0.86
(nmol/mg protein)	no food	4.43 ± 0.70	3.79 ± 0.52	0.65 ± 0.33	6.25 ± 0.52
Spermine	control	0.96 ± 0.25	1.23 ± 0.36	0.81 ± 0.29	4.90 ± 0.61
(nmol/mg protein)	no food	0.88 ± 0.33	1.18 ± 0.39	0.94 ± 0.24	5.14 ± 0.67

The experimental procedure was as reported in the 'Materials and methods' section. The values are the means $\pm$ SEM of six determinations. Statistical comparisons were made using Student's t-test.

significantly increased putrescine level in liver, brain, heart and muscle, without significantly modifying spermine and spermidine content. Cold exposure also markedly increased the activity ODC, the key enzyme in the biosynthesis of putrescine. Seasonal changes in polyamine concentrations have been reported in different tissues from the sea bass (*Dicentrarchus labrax* L.) [17], with an increase in putrescine content and a decrease in spermidine and spermine during the winter. However, the variations in enzyme activity and polyamine concentration measured in liver from goldfish at different times of the year [15] appeared temperature-independent, suggesting that other factors, probably related to seasonal changes in fish metabolism, interact with temperature to determine stimulation of hepatic ODC. Yet our findings are in contrast with the stimulatory effect of water temperature on ODC activity and polyamine content observed by Neyfakh et al. [16] in loach (*Misgurnus fossilis*) embryos.

ODC activity seems also to depend on the nutritional status of fish. A strong reduction of hepatic ODC activity in brook trout was observed during short-term starvation [21], and variation in enzyme activity, as a function of changes in diet protein composition and water temperature, have been reported in goldfish liver [22]. Nevertheless, we failed to demonstrate any correlation between starvation and ODC activity.

Kinetic studies can also elucidate the mechanisms by which temperature elicits perturbations in the ontogeny of ODC and influences polyamine metabolism. In brain from goldfish exposed to cold, a K_m resembling the control value was obtained, but the V_{max} of the enzyme was eight times higher than that of control fish. Effects on V_{max} rather than on K_m account for the increase in brain ODC activity in fish exposed to cold. Previous studies on the effects of cold exposure on activities of enzymes in ectothermic animals suggest that a basic mechanism of acclimation involves the production of higher quantities of enzymes in order to compensate for decreases in temperature [23, 24]. In view of the fact that cold exposure decelerates protein synthesis and cellular maturation in general [25, 26], it is unlikely that the observed increase in ODC activity is due to de novo synthesis of new enzyme. Most likely, the increase in activity is due to either activation or deinhibition of existing enzyme.

Increase of ODC activity, followed by transient accumulation of its product putrescine, is one of the first metabolic events detectable in many biological systems under the action of a number of stressors [27]. In epidermal cells of teleost fish, exposure to cold has been shown to cause dissociation of intermediate filaments that is readily reversed upon rewarming the cells [28]. As in mammals, putrescine seems implicated in promoting microtubule assembly in the early stages of regeneration [29]. Changes in putrescine concentration and in ODC activity observed in various tissues from goldfish following exposure to low temperature may be related to the arrangement of the cytoskeleton as part of the adaptive response evoked by environmental modifications.

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