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Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress

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Abstract

Levels of lipid peroxides (LOOH), thiobarbituric-acid reactive substances (TBARS), protein carbonyls and low- and high-molecular weight thiols were measured in brain, liver, kidney, and white muscle of goldfish, *Carassius auratus* L., over 1–12 h of high temperature (35 °C) exposure followed by 4 or 24 h of lower (21 °C) temperature recovery. LOOH and TBARS contents increased during heat shock exposure with a maximal rise of 20-fold for liver TBARS, but both mainly reversed at recovery. Protein carbonyl content was unaffected by heat shock but rose in brain, liver, and kidney during recovery. Low-molecular weight thiol concentrations unexpectedly increased up to ~4-fold in brain, kidney and muscle under heat shock and remained high during recovery. Protein thiol contents also rose in liver and muscle during high temperature exposure by 2- and 3-fold, respectively, and decreased to control values or below in all tissues at late recovery. Low- and high-molecular weight thiol levels inversely correlated in liver ($R^2=0.87$) suggesting that the former was used to reduce the latter over the experiment. It is concluded that the redox balance in goldfish tissues is strictly maintained probably contributing to the high tolerance of this species to heat shock.

Keywords: Carbonylproteins; Goldfish; Heat shock; Lipid peroxides; Oxidative stress; Thiobarbituric-acid reactive substances; Thiols

1. Introduction

Ectothermal animals can be exposed to episodes of sharp temperature changes often called heat shock. As shown on starry flounder, *Platichthys stellatus*, an increment of temperature results in an increase in oxygen uptake with an increase in ventilation volume, cardiac output, ventilation–perfusion ratio and, on the other hand, leads to a decrease in the effectiveness of oxygen removal, transfer factor and arterial and venous oxygen content (Watters and Smith, 1973). Meanwhile, some aquatic ectotherms experience hypoxia at high environmental temperatures (Downing and Merkens, 1957). Long-term adaptations to high temperatures result in crucial modifications of intermediary metabolism and cell membrane properties (Cossins, 1981). However, a sharp temperature increase can cause heat shock stimulating numerous changes. In this case the main attention is paid to so-called heat shock proteins (HSP) operating as cellular "chaperones" and having clear protective effects (Feder and Hofmann, 1999). An increase of environmental temperature which leads to metabolic activation combined with an increase in oxygen consumption initiates the so-called oxidative stress (Halliwell and Gutteridge, 1989). The term "oxidative stress" is used for states where the balance between generation and elimination of reactive oxygen species (ROS; superoxide anion, O_2^-, hydrogen peroxide, H_2O_2, and hydroxyl radical, $^{\bullet}OH$) is disturbed in favour of the former (Sies, 1991). Even mild oxidative stress may have serious deleterious effects modifying many cellular functions up to death. Responding to these problems cells subjected to heat shock increase the antioxidant defenses particularly the so-called antioxidant and associated enzymes (Hermes-Lima, 2004). The final result of the above-mentioned events will depend on the ability of an organism to quickly raise antioxidant potential in response to oxidative stress.

Little is known about oxidative stress in fish exposed to heat shock. Studies in this area are mainly directed to HSP induction and characterization in fish cell culture or whole organisms under different stressful conditions (Martin et al., 1998; Ohnishi

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et al., 1998; Kagawa, 2004; Kondo et al., 2004) or physiological adaptations and gene expression during thermal acclimation to lower or higher temperatures (Jürss et al., 1987; Parihar and Dubey, 1995; Malek et al., 2004). Adaptations of temperature-tolerant species are of a particular interest. The goldfish, *Carassius auratus* L., is an eurythermal species being able to survive static temperatures between about 0 and 41 °C and short-term exposures to temperatures close to 44 °C (Love, 1970; Ford and Beitinger, 2005). Besides, the species is highly resistant to other kinds of stresses such as anoxia, hypoxia and hyperoxia (van den Thillart and van Waarde, 1985; Lushchak et al., 2001, 2005a). This work was designed to give a wide picture of goldfish responses to a sharp temperature increase and recovery including changes of both levels of oxidatively damaged proteins and lipids, thiol content and activities of antioxidant enzymes. We aimed to clarify which elements of goldfish antioxidant system are responsible for the tolerance of heat shock. Here we describe the effects of temperature transitions from 21 to 35 °C and recovery to the initial temperature (21 °C) on oxidative damage of different cellular components—lipids, proteins, and thiols.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene, ethylenediamine-tetraacetic acid (EDTA), ferrous sulphate, xylenol orange, cumene hydroperoxide, thiobarbituric acid, 2,4-dinitrophenylhydrazine, 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich Chemical Co. (USA). All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Goldfish (*C. auratus* L.) of both sexes weighing 20–50 g were purchased at a local fish farm (Burshtyn) and kept in dechlorinated tap water and fed with standard fish food. Temperature was maintained at 21±1 °C with a natural light–dark cycle with light from about 6:00 to 20:00 h. Goldfish were held under these conditions for at least two weeks before experimentation.

For experimentation, fish were carefully transferred to 100 L aquaria placed in a thermostatted room with the water temperature in aquaria of 35±1 °C and held for 1, 6 or 12 h with aeration. After indicated periods, fish were sampled for organ dissection, six specimens per each group. Other groups of fish experienced 12 h heat shock were returned to the initial temperature, 21±1 °C, and sampled after 4 or 24 h of recovery. Control fish were taken from an aquarium with the temperature of 21±1 °C. The oxygen content was 8.2–8.8 mg/L in aquaria with 21 °C and decreased at 35 °C to 6.1–6.5 mg/L; hence, goldfish were under normoxic conditions over the whole experiment. Oxygen concentration in the water was measured by the Winkler method (Lurje and Rybnikova, 1974). For sampling, fish were killed by trans-spinal dissection and the brain, liver, kidneys and white muscle were quickly removed. The samples were immediately used for measuring the parameters of interest.

2.3. Indices of oxidative stress

Tissue samples were homogenized (1 : 10 w/v) using a Potter-Elvehjem glass homogenizer in 50 mM potassium phosphate (KPi) buffer, pH 7.0, containing 0.5 mM EDTA and a few crystals of phenylmethylsulfonyl fluoride, a protease inhibitor. A 250 μL aliquot of this homogenate was then mixed with 0.5 mL of 10% (final concentration) trichloroacetic acid (TCA) and centrifuged for 5 min at 13,000 ×*g*. Carbonylprotein (CP) levels were measured in the resulting pellets, and thiobarbituric-acid reactive substances' (TBARS) contents were assayed in the supernatants using a spectrophotometer SF-46 (LOMO, USSR).

Carbonyl derivatives of proteins were detected by reaction with 2,4-dinitrophenylhydrazine (DNPH) as described by Lushchak et al. (2005a) except for using 6 M urea instead of guanidine–HCl to dissolve protein pellets. Resulting 2,4-dinitrophenylhydrazones were quantified spectrophotometrically at 370 nm using a molar extinction coefficient of 22×10^3 M^{-1} cm^{-1} (Lenz et al., 1989). The amounts of CP were expressed as nmol of CP per gram wet mass (gww) of tissue.

The end products of lipid peroxidation, including malondialdehyde, were measured by the TBARS assay (Lushchak et al., 2005a). Absorption at 535 nm and a molar extinction coefficient of 156×10^3 M^{-1} cm^{-1} were used to calculate TBARS concentrations (Rice-Evans et al., 1991). The values are expressed as nmol of TBARS per gram wet mass of tissue.

Lipid peroxides (LOOH) are among initial products of lipid peroxidation. LOOH content was measured with xylenol orange (Hermes-Lima et al., 1995) with minor modifications (Lushchak et al., 2005a). The LOOH levels are expressed as nmol of cumene hydroperoxide equivalents per gram wet mass of tissue.

Free thiols are widely measured by the Ellman procedure with DTNB (Ellman, 1959). Briefly, to measure total thiol content (low and high molecular weight thiols), 50 μL of supernatants obtained as described above were incubated with 20 μM DTNB in 50 mM KPi buffer, pH 8.0, for 30 min. Controls contained distilled water instead of supernatant. Absorption was read at 412 nm and a molar extinction coefficient of 14×10^3 M^{-1} cm^{-1} was used for calculation of thiol concentration. For measuring low-molecular weight thiol (L-SH) content, 50 μM of supernatant were mixed with 25 μL of 10% (final concentration) TCA, centrifuged for 5 min at 13,000 ×*g* and the whole supernatant was used for assay. In this case, control samples contained the same volume of TCA. The thiol levels are expressed as micromoles of SH-groups per gram wet mass of tissue. The high-molecular weight thiol (H-SH) content was calculated by subtracting the L-SH concentration from the total thiol level.

2.4. Statistics

Data are presented as means±SEM. Statistical analysis was performed using analysis of variance (ANOVA) followed by a Student–Newman–Keuls test. Correlation analysis was performed using a computer statistical program MYNOVA