Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1052 (2005) 180 – 186

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research Report

Catalase inhibition by amino triazole induces oxidative stress in goldfish brain

Tetyana V. Bagnyukova[a], Olena Yu. Vasylkiv[a], Kenneth B. Storey[b], Volodymyr I. Lushchak[a,*]

[a]*Department of Biochemistry, Institute of Natural Sciences, Vassyl Stefanyk Precarpathian National University, 57 Shevchenko Street, 76025, Ivano-Frankivsk, Ukraine*

[b]*Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6*

Accepted 5 June 2005
Available online 14 July 2005

Abstract

The effects of in vivo inhibition of catalase by 3-amino 1,2,4-triazole (AMT) on the levels of damage products resulting from reactive oxygen species attack on proteins and lipids as well as on the activities of five antioxidant and associated enzymes were studied in the brain of goldfish, *Carassius auratus*. Intraperitoneal injection of AMT at a concentration of 0.1 mg/g wet weight caused a gradual decrease in brain catalase activity over 72 h, whereas higher AMT concentrations (0.5 or 1.0 mg/g) reduced catalase activity by about two-thirds within 5–10 h. AMT effects on antioxidant enzyme activities and oxidative stress markers were studied in detail using fish treated with 0.5 mg/g AMT for 24 or 168 h. The levels of thiobarbituric acid-reactive substances (a lipid damage product) increased 6.5-fold by 24 h after AMT injection but fell again after 168 h. The content of carbonylproteins (CP) also rose within 24 h (by ~2-fold) and remained 1.5-fold higher compared with respective sham-injected fish after 168 h. CP levels correlated inversely with catalase activity (R^2 = 0.83) suggesting that catalase may protect proteins in vivo against oxidative modification. The activities of both glutathione peroxidase and glutathione-*S*-transferase increased by ~50% and 80%, respectively, in brain of AMT-treated fish and this might represent a compensatory response to lowered catalase activity. Possible functions of catalase in the maintenance of prooxidant/antioxidant balance in goldfish brain are discussed.

Theme: Other systems of the CNS
Topic: Brain metabolism and blood flow

Keywords: Goldfish; Catalase; 3-Amino 1,2,4-triazole; Glutathione peroxidase; Carbonylprotein

1. Introduction

Most living organisms depend on ATP generation by oxygen-based metabolism, but one consequence of oxygen dependence is the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$), mainly as byproducts of oxidative metabolism. The mitochondrial electron transport chain and a variety of cellular oxidases are the main sources of ROS generation [38]. ROS can attack multiple cellular constituents, including proteins, nucleic acids, and lipids. To cope with the damaging actions of ROS, organisms have evolved multiple systems of antioxidant defense. So-called low-molecular weight antioxidants include metabolites such as glutathione, ascorbic acid, tocopherol, uric acid, etc., whereas high-molecular weight defenses include enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione-*S*-transferase (GST) [12,14,17,24]. These enzymes that deal directly with radical species and the damage caused by them to macromolecules constitute the first line of antioxidant enzymatic defense, whereas other enzymes including glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) contribute to the renewal of reducing power. GR catalyzes

* Corresponding author. Fax: +1 38 03422 31574.
E-mail address: lushchak@pu.if.ua (V.I. Lushchak).

0006-8993/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.brainres.2005.06.002

the NADPH-dependent reconversion of oxidized glutathione to reduced GSH, whereas G6PDH is a primary source of NADPH synthesis.

Under normal physiological conditions, a relatively low steady-state level of ROS in cells is the result of a balance between ROS formation in prooxidant processes and ROS elimination by enzymatic and non-enzymatic scavengers. Constitutive activities of antioxidant enzymes are coordinated to ensure the optimal defense against exo- and endogenic ROS generation. Each enzyme carries out specific functions, although an overlap between some enzymes may exist; for example, both catalase and GPx can degrade hydrogen peroxide [15,17,32]. The roles of these two antioxidant enzymes in physiological defense against peroxides are still not understood in full. Some studies suggested that catalase was the main H_2O_2-detoxifying enzyme [3,4,8,21], whereas other investigations found that GPx was more efficient at H_2O_2 detoxification compared to catalase [11,34]. One approach that can be used to elucidate the physiological functions of antioxidant enzymes in cells/organs is to modify their content. For example, a controlled decrease in the amount of catalase activity can be achieved by administering 3-amino 1,2,4-triazole (AMT), an irreversible inhibitor of catalase [30].

Brain has intrinsically low to moderate activities of catalase and GPx, whereas SOD is prevalent in normal brain tissue [14,15,28,29]. Brain is an organ in which homeostasis must be strictly maintained, based on a high dependence on oxidative phosphorylation. Since a major source of ROS is the leakage of electrons from the electron transport chain, organs with a high dependence on ATP generation by oxidative phosphorylation need effective ways to detoxify O_2^- and H_2O_2. On the other hand, certain level of ROS is needed as signaling molecules involved in normal functioning of brain, and they may modify the signal transduction pathways [7]. This study aimed to investigate the effect of catalase inhibition by AMT on the antioxidant enzyme defenses and oxidative damage to proteins and lipids in goldfish brain in order to clarify the role of catalase in the protection of tissue proteins against ROS attack and illuminate possible mechanisms that could substitute for catalase function when the enzyme was inhibited.

2. Materials and methods

2.1. Chemicals

Phenylmethylsulfonyl fluoride (PMSF), butylated hydroxytoluene (BHT), yeast glutathione reductase (GR), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), glucose-6-phosphate (G6P), ethylenediamine-tetraacetic acid (EDTA), sodium azide, and 3-amino 1,2,4-triazole (AMT) were purchased from Sigma Chemical Co. (USA). *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), $NADP^+$, and NADPH were from Reanal (Hungary), Tris–HCl was from Bio-Rad, and guanidine–HCl was from Fluca. All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Goldfish (*Carassius auratus* L.) of both sexes weighing 20–70 g were purchased at a local fish market (Ivano-Frankivsk, Ukraine) and were kept in dechlorinated tap water and fed with standard fish food. Temperature was maintained at 18 ± 1 °C with a natural light–dark cycle with light from about 8:00 am to 5:00 pm. Goldfish were acclimated to these conditions for at least 1 month before experimentation.

For preliminary investigations of the effect of AMT on catalase inhibition, fish were injected intraperitoneally with AMT diluted in physiological saline (0.9% NaCl) at final concentrations 0.1, 0.5, or 1.0 mg/g wet body weight (gww). The volume of injected solution was 0.45% of body weight. Controls were injected with 0.9% NaCl alone. Fish were killed by transspinal dissection at 5, 10, 24, 48, 72, 120, or 168 h after the treatment. The brain was quickly removed and used immediately to measure catalase activity.

For the next study, fish were injected with AMT solution at a final concentration of 0.5 mg/gww (the volume injected was 0.3% of body weight). Another group of goldfish was treated with the same volume of 0.9% NaCl. Control fish were not treated. After 24 or 168 h, fish injected with AMT or NaCl solutions were sampled, and brain tissue was processed immediately to measure the parameters of interest.

2.3. Indices of oxidative stress

Tissue samples were homogenized (1:10 w/v) using a Potter–Elvjeham glass homogenizer in 50 mM potassium phosphate (KPi) buffer, pH 7.0, containing 0.5 mM EDTA and a few crystals of PMSF, 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* in an Eppendorf centrifuge. Carbonylprotein (CP) levels were measured in the resulting protein 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) [20]. Resulting 2,4-dinitrophenylhydrazones were quantified spectrophotometrically in guanidine chloride solution which was used to solubilize protein pellets. The amount of CP in the resulting supernatants was evaluated at 370 nm using a molar extinction coefficient of 22×10^3 M^{-1} cm^{-1} [20]. The values were expressed as nanomoles of CP per protein milligram in the guanidine chloride solution.

The decomposition of lipid hydroperoxides produces low-molecular weight products, including malondialdehyde, which can be measured by the TBARS assay [35]. Malondialdehyde and other aldehydes when boiled with