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Comparative Biochemistry and Physiology, Part C 143 (2006) 67 – 72
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Metabolical changes induced by chronic phenol exposure in matrinxã *Brycon cephalus* (teleostei: characidae) juveniles

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Received 17 August 2005; received in revised form 13 December 2005; accepted 18 December 2005
Available online 3 February 2006

Abstract

Phenol and its derivatives are xenobiotics present in many industrial wastewaters and in non-specific pesticides. It is a lipophilic compound and, therefore, accumulates along the trophic chain. Phenol is often found in marine and fresh water environments. The aim of this work was to detect metabolic changes induced by phenol in *Brycon cephalus* juveniles. Several enzymes activities and metabolites were quantified in the liver, white muscle and plasma. Among the enzymes assayed are alanine and aspartate amino transferases (ALAT and ASAT), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH). Glucose, glycogen, lactate, ammonia and pyruvate were also quantified in tissues and plasma (glycogen in tissues only). The liver was the most responsive organ. The activities of the transaminases increased in muscle and liver, followed by an increase in hepatic ammonia. Correlation between ammonia and transaminases points towards phenol-induced consumption of protein. Hepatic glycogen and glucose contents were lower followed exposure to phenol. The same was observed for muscle glucose, suggesting considerable use of carbohydrate stores. The activity of hepatic lactate dehydrogenase increased with negative correlation with muscle lactate. This suggests that hepatic gluconeogenesis supplies tissues like muscle and brain with glucose. These results indicate that phenol intoxication demands metabolic energy and leads to significant changes of the metabolic profile of the fish, inducing to a certain extent a shift from carbohydrate catabolism to protein catabolism and the activation of gluconeogenesis.

Keywords: *Brycon cephalus*; Phenol; Metabolism; Protein; Carbohydrate; Enzymes; Stress; Pollution

1. Introduction

Among the many stressful environmental factors to which animals are subjected to, xenobiotics have become more and more relevant due to anthropogenic impact on the environment. Industrial and domestic wastes have been dumping a wide range of chemicals to natural habitats. Phenol and its derivatives are common substances present in industrial wastewaters and in non-specific pesticides, herbicides, bactericides and fungicides (Gupta et al., 1983). In Brazil's inland waters phenol concentrations are often found to be above the permitted levels (CETESB, 2003). Moreover, it is also commonly found in marine environments and in fish tissue (Mukherjee et al., 1990). Phenol is highly lipophilic and the absorption of its chloride derivatives occurs through passive diffusion of non-ionic forms (Kishino and Kobayashi, 1995). Its mechanisms of action are multiple and often antagonistic (Roche and Bogé, 2000). Some effects reported include: genotoxic (Jagetia and Aruna, 1997); carcinogenic (Tsutsui et al., 1997) and immunotoxic effects (Taysse et al., 1995). In contrast, at times, phenols may act as free radicals scavengers and prevent genetic damage caused by other agents (Stich, 1991). Because of its lipophilicity, phenol has a potential for accumulating along the trophic chain. Therefore, phenol not only presents a threat to natural environment, but also to human health. Phenol intoxication must be considered in the fish rearing systems. Saha et al. (1999) demonstrated that when phenol is present in the water, food consumption, mean weight and fertility are significantly reduced in fish.

These effects at the organismal level can be a result of many actions of phenol beyond those referred above. For example, Dunier and Siwicki (1993) reported that phenol causes suppression on fish immune system. It can also cause substantial changes in the plasma membrane phospholipid composition (Kotkat et al., 1999). Moreover, phenol and its derivatives can

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1532-0456/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2005.12.004

cause many alterations on the metabolism of fish (Holmberg et al., 1972; Dalela et al., 1980; Gupta et al., 1983; Reddy et al., 1993). Many enzymes of intermediary metabolism of fish are affected by exposure to phenol. Gupta et al. (1983) found both ALAT and ASAT activities altered in different tissues by a wide range of phenolic compounds. Other enzymes such as succinate dehydrogenase (SDH), LDH, acetyl cholinesterase (AChE) and glutamate dehydrogenase (GDH), were found to respond to phenol intoxication in the brain and white muscle of *Channa punctatus* (Reddy et al., 1993). Other reports have pointed out other enzymes susceptible to phenol such as alkaline and acid phosphatases (Dalela et al., 1980), superoxide dismutase (SOD) and catalase (Roche and Bogé, 1996). These results indicate the phenol can affect carbohydrate metabolism by, for example, altering the inter-conversion of lactate into pyruvate by LDH. It can also alter protein metabolism by altering transamination of amino acids by ALAT and ASAT or the production of ammonia by GDH. Changes in the activity of SDH can affect the balance between anaerobic and aerobic metabolism. Therefore, the sum of these alterations can have a significant effect on energy metabolism.

Brycon cephalus (matrinxã) is a Brazilian neotropical fish originating from the Amazonian basin. According to Castagnolli (1992) this species has achieved a considerable rearing success because of its accelerated growth rate and good acceptance of commercial food. In some regions, deforestation, dams and water pollution are the main threat to this particular fish (Mendonça, 1996). Therefore, understanding the mechanisms of toxicological actions of phenol becomes an important data for conservation, especially when no related studies with this pollutant, concerning the effects on Neotropical fish, are available. Thus, the objective of this work was to detect alterations in energy metabolism of *B. cephalus* induced by the exposure to environmental phenol (2 mg/L; 10% of LC-50/96 h) to gain a better understanding of the organismal effects of this chemical and the adaptive metabolic responses of this species.

2. Material and methods

2.1. Experimental design

B. cephalus Gunther 1869 (Teleostei: Characidae) (length: 19.9±0.2 cm, mass: 112.6±4.1 g) were obtained from the commercial fish farm "Águas Claras" in Mococa-SP, Brazil. The fish remained in 1000 L tanks for 2 weeks for acclimation. Then, they were divided into four tanks, each tank containing 14 fish. There, the fish remained with natural photoperiod and constant temperature (24 °C) for 48 h. The experiment was carried out in duplicates. Therefore, after the acclimation period, 2 mg per L of phenol (10% of the LC-50/96 h for *B. cephalus*, previously assayed in our laboratory) was added to two of the tanks (experimental) and the other two remained as controls. The water of the whole system was renewed every 12 h (in this period 30% of the phenol is lost due to volatilization) and phenol was again added to the experimental tanks. Water quality parameters were analyzed throughout the whole experiment (total alkalinity 52.08±2.49 mg/L as $CaCO_3$; hardness 49.88±3.29 mg/L as $CaCO_3$; pO_2 5.97±0.15 mg/L; temperature 23.79±0.39 °C; pH 7.60±0.03; conductivity 123.00±8.10 μS cm^{-3}; ammonia 0.28±0.04 mg/L; nitrite 15.28±4.26 μg/L). At the end of the 96 h period, ten fish were collected from each tank. Blood was collected with heparinized syringes. Fish were then anaesthetized with MS-222 and sacrificed for the sampling of white muscle and liver. These samples were immediately frozen in liquid nitrogen alongside with the plasma obtained through the centrifugation (21,000 ×*g* for 3 min) of whole blood.

2.2. Enzymatic assays

Tissues for enzyme assays were homogenized at 4 °C in buffer consisting of 50% glycerol and 50% 10 mM (pH 7.0) phosphate buffer. The homogenates were centrifuged twice (600 ×*g* for 3 min; 6000 ×*g* for 8 min, both at 4 °C) and the final supernatant was used for the assays. For plasma enzymes, whole plasma was used. In the white muscle the activities of LDH (5.0 mM pyruvate, 0.1 mM NADH and 42.5 mM Tris buffer, pH 7.5) and malate dehydrogenase (MDH)(0.33 mM oxaloacetate, 0.2 mM NADH and 50 mM imidazole buffer, pH 7.0) were determined kinetically at 340 nm through the consumption of NADH (Hochachka et al., 1978). Pyruvate kinase (PK) was determined kinetically at 340 nm, using NADH, in a coupled reaction with LDH (2.8 mM phosphoenolpyruvate, 100 mM KCl, 100 mM MgCl, 0.15 mM NADH, 21 U/ml LDH and HEPES buffer, pH 7.5) (Staall et al., 1975). ALAT and ASAT (222 mM alanine or 44.4 mM aspartate, 11.6 mM α-ketoglutarate, 0.22 mM arsenate, 0.27 mM pyridoxal phosphate and phosphate buffer, pH 7.0) were determined colorimetrically at 430 nm with the end-point method described by Reitman and Frankel (1957). In liver, glutamate dehydrogenase (GDH) (250 mM ammonium acetate, 5.0 mM α-ketoglutarate, 1.0 mM ADP and 50 mM imidazole buffer, pH 7.0) activity was also determined kinetically at 340 nm (Hochachka et al., 1978). In the plasma only ALAT, ASAT and LDH were measured.

2.3. Metabolic intermediates

The homogenates used for the metabolites were either result of protein precipitation with 20% trichloroacetic acid, followed by centrifugation (21,000 ×*g* for 3 min) or homogenization in water, followed by the same centrifugation. The supernatants were used as samples for the analyses. Intermediates determined in white muscle and liver were: ammonia using Nessler's solution (Gentzkow and Masen, 1942); lactate by its reaction with *p*-nitrophenylphenol (Harrower and Brown, 1972); pyruvate by its reaction with dinitrophenylhydrazine (Lu, 1972); glucose by the glucose oxidase reaction (Trinder, 1969); glycogen by alkaline digestion of the tissue and alcoholic precipitation (Bidinotto et al., 1997), followed by the determination of the reducing sugar contents by Dubois et al. (1956) method and free amino acids by reaction with ninhydrin (Copley, 1941). In plasma the same parameters were analyzed with