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Carbaryl exposure and recovery modify the interrenal and thyroidal activities and the mitochondria-rich cell function in the climbing perch *Anabas testudineus* Bloch

Valsa S. Peter[a], G.S. Babitha[a], S.E. Wendelaar Bonga[b], M.C. Subhash Peter[a,*]

[a] Department of Zoology, University of Kerala, Kariavattom, Thiruvananthapuram 695 581, Kerala, India
[b] Department of Animal Ecology and Ecophysiology, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

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ABSTRACT

We examined the effects of carbaryl (1-naphthyl methylcarbamate; sevin), a carbamate pesticide, on interrenal and thyroid activities and mitochondrial rich (MR) cell function in climbing perch to understand the physiological basis of toxicity acclimation in this fish to the chemical stressor. Carbaryl exposure (5–20 mg L^{-1}) for 48 h increased cortisol and glucose, but decreased the T_3 level without affecting T_4 concentration in the plasma. These responses of the carbaryl-exposed fish were nullified and a rise in plasma T_4 occurred in these fish when they were kept for 96 h recovery in clean water. A tight plasma mineral control was indicated in the carbaryl-exposed fish as reflected by the unchanged plasma Na, K, Ca and inorganic phosphate levels. The ouabain-sensitive Na^+, K^+-ATPase activity showed an increase in the gills but the intestinal and renal tissues showed little response to carbaryl treatment. However, substantial increases in the intestinal and renal Na^+, K^+-ATPase activities occurred in the recovery fish. The MR cells in the branchial epithelia showed a strong Na^+, K^+-ATPase immunoreactivity to carbaryl treatment indicating an activated MR cell function. The numerical MR cell density remained unchanged, but stretching of secondary gill lamellae as part of gill remodeling occurred during carbaryl exposure. The increased surface of these lamellae with abundant MR cells as a result of its migration into the lamellar surface points to marked structural and functional modifications of these cells in the carbaryl-treated fish which is likely to a target for carbaryl action. The rise in plasma T_4 and the restoration of normal branchial epithelia in the recovery fish indicate a thyroidal involvement in the recovery response and survival. Our data thus provide evidence that carbaryl exposure and its recovery evoke interrenal and thyroid disruption in this fish leading to a modified osmotic response including an altered MR cell function.

1. Introduction

Indiscriminate pesticide use in agriculture and the resulting widespread distribution of pesticide residues evoke a threat to non-target organisms including fishes. The neurotoxic pesticide N-methyl carbamate like carbaryl, is known for its inhibitory action in acetyl cholinesterase system that can impose adverse effects on many physiological processes of fish (Tierney et al., 2006; Kavitha and Rao, 2007; Mdegela et al., 2010). Because of the vulnerability of fish to aquatic toxicants, many of these chemical stressors have frequently been shown to disrupt their water and ion regulation (Leji et al., 2007; Lock and Wendelaar Bonga, 2008; Adeyemi et al., 2012). Similarly, exposure of fishes to pesticides (Sancho et al., 1997; Hontela et al., 2008; Lock and Wendelaar Bonga, 2008; Peter et al., 2009), acids (Brown et al., 1990; McCormick et al., 2009; Peter and Rejitha, 2011), industrial waste (Leji et al., 2007; Peter et al., 2007), ammonia, and metals (Dang et al., 2001; Chowdhury and Wood, 2007) induces changes in the branchial epithelium and alters the activity of Na^+, K^+-ATPase thereby modifying the normal flow of ions. Acute and chronic exposures of fish to insecticides affect plasma ion concentrations (Mishra et al., 2001; Singh et al., 2002) and ion transporting ATPases including Na^+, K^+ ATPase (Kakko et al., 2003; Agrahari et al., 2007).

Fishes possess complex neuroendocrine mechanisms which allow them to combat the disturbed physiological homeostasis particularly during the exposure to chemical stressors (Wendelaar Bonga, 1997; Iwama et al., 2006; Peter et al., 2007; Schreck, 2010; Peter and Peter, 2011). Changes in hydromineral processes and energy metabolism are important adaptive modifications that fish show during stress (Peter et al., 2004, 2007, 2009; Lock and Wendelaar Bonga, 2008; Adeyemi et al., 2012; Peter and Peter, 2011). Cortisol, the end product of corticosteroidogenesis that

* Corresponding author. Tel.: +91 471 241 8906; fax: +91 471 259 7514.
E-mail address: subashpeter@yahoo.com (M.C.S. Peter).

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occurs in the interrenal cells of head kidney of teleostean fishes, has been associated with metabolic and hydromineral control (Vijayan et al., 1997, 2003; Dang et al., 2000). This primary stress hormone in teleosts that stimulates the synthesis of energy substrates (Vijayan et al., 1997), and many other physiological processes (Wendelaar Bonga, 1997; Mommsen et al., 1999; Babitha and Peter, 2010), shows sensitivity to many endocrine disrupting chemicals (Hontela, 2005; Lock and Wendelaar Bonga, 2008). Similar to the interrenals, the thyroid gland that releases thyroxine (T_4) and triiodothyronine (T_3) as the primary thyroid hormones (THs), is also involved in the regulation of a wide range of biological processes in fishes, including energy metabolism and hydromineral regulation (Peter et al., 2000, 2011; Power et al., 2001; Arjona et al., 2008; Peter and Peter, 2009). Thyroid function in fish often shows sensitivity to many environmental variables and chemical stressors including endocrine disrupting chemicals (Leatherland, 1994; Peter et al., 2007; Peter and Rejitha, 2011; Feng et al., 2012) and now it is clear that THs are involved in stress response of fish (Peter, 2011; Peter and Peter, 2011).

Gills, the main sites for gas exchange, hydromineral regulation and nitrogenous excretion, are especially vulnerable to aquatic contaminants including pesticides (Evans et al., 2005; Lock and Wendelaar Bonga, 2008; Peter and Rejitha, 2011). The resulting disturbance may trigger physiological and morphological alterations that will lead to restoration of branchial functions (Evans et al., 2005; Lock and Wendelaar Bonga, 2008). Exposure of fishes to pesticides leads to gill lesions including hyperplasia and thrombosis in the secondary lamellae (Rao et al., 2005). Mitochondria-rich cells (MR) in the branchial epithelia that possess Na^+, K^+-ATPase, the biochemical equivalent to the sodium pump, is a target cell for aquatic toxicants (Li et al., 1998; Dang et al., 2000). The activity of this enzyme that serves as a biomarker to contaminants is sensitive to many pesticides and industrial effluents (Dang et al., 2000; Leji et al., 2007). For example, a reduction in the gill Na^+, K^+-ATPase activity was observed in European eels (*Anguilla anguilla*) treated with fenitrothion or thiobencarb (Sancho et al., 1997, 2003) or monocrotophos (Agrahari et al., 2007). On the contrary, in climbing perch an upregulation of Na^+, K^+-ATPase activity occurred when exposed to coconut husk retting effluents (Leji et al., 2007) or kerosene (Peter et al., 2007).

As the process of accommodation of environmental and biological challenges, acclimation imposes physiological and structural modifications in fishes (Peter and Peter, 2011; Peter and Rejitha, 2011). Toxic acclimation of fish to chemical stressors is complex and it is likely that changes in the interrenal and thyroid activities followed by the compensatory hydromineral modification might contribute to this process (Leji et al., 2007; Peter et al., 2007, 2009; Lock and Wendelaar Bonga, 2008; Peter and Peter, 2011). It is hypothesized that in response to pesticides, fish may modify their osmotic competence, branchial structure and MR cell function and alter hormones of thyroid and interrenal glands. The purpose of the study was to test this hypothesis in the climbing perch *Anabas testudineus* after exposing them to carbaryl, a carbamate pesticide which is moderately toxic to aquatic organisms including fishes (Tierney et al., 2006; Ferrari et al., 2007; Mdegela et al., 2010).

2. Materials and methods

2.1. Animals

Tropical freshwater air-breathing fish commonly known as climbing perch (*A. testudineus* Bloch) belonging to order Perciformes and family Anabantidae was used as the test species. This native teleost fish inhabiting in the backwaters of Kerala in Southern India is an obligate air-breathing fish equipped to live in demanding environmental conditions with its well-defined physiological and biochemical mechanisms (Peter et al., 2007, 2011). These fish in their post-spawning phase were collected from the wild and held in the laboratory conditions for three weeks under natural photoperiod (12 hL:12 hD) and at water temperature ranging from 28 to 29° C with a mean water pH of 7.2. Fish were fed with dry commercial fish feed at 1.5% of body mass/day and were transferred to 50 L glass tanks for static experimentation. There was no mortality during experimentation and the fish ate their meals. The regulations of Animal Ethical Committee of the University were followed.

2.2. Protocol and sampling

Two experiments were conducted. In the first, the laboratory acclimated fish groups in static system were exposed to two sublethal concentrations (5 and 20 mg L^{-1}) of carbaryl (1-naphthyl methylcarbamate; Sevin, Bayer) for 48 h. A separate fish group without carbaryl treatment served as control (0 concentration). In the second experiment, two fish groups were exposed to 20 mg L^{-1} of carbaryl for 48 h. While one group was sampled after carbaryl exposure for 48 h, the other group was held in clean freshwater for 96 h to recover from carbaryl exposure. An untreated fish group (0 concentration) was also held. The aquaria water was slowly replenished with freshwater with appropriate carbaryl concentration on the second day and no mortality was recorded. All treatments were done concurrently to avoid interaction with other environmental variables. There was no specific behavioral change observed in the treated fish which ate their normal food ration.

After exposure, the fish were anaesthetized in 0.1% 2-phenoxyethanol solution. Blood was drawn by caudal puncture with heparinized syringe and centrifuged at 5000 g for 5 min at 4 °C and plasma was separated and stored at −20 °C until analysis. The fish were sacrificed by spinal transsection and gills, intestine and kidney were excised and placed in ice cold 0.25 M SEI buffer (pH 7.1) and stored at −20 °C. A few gill arches were collected and fixed in Bouin's fixative for 24 h.

2.3. Analyses

2.3.1. Plasma glucose, cortisol, T_3 and T_4

Plasma glucose was determined colorimetrically using GOD/POD test kits (Span Diagnostics Ltd., New Delhi). Cortisol concentrations in plasma samples were measured by competitive immunoenzymatic assay (DiaMetra, Foligno, Italy) and the values were expressed as ng ml^{-1}. The sensitivity and reliability of this method was examined and the values were comparable to a RIA method reported earlier (Peter and Peter, 2007). In brief, plate wells coated with anti-cortisol (mouse-anti-rabbit IgG) were treated with standards and diluted deproteinized plasma samples, and incubated with 200 μL cortisol-HRP conjugate at 37 °C for 1 h. After washing, 100 μL tetramethyl benzidine (TMB-H_2O_2) was added and incubated at 20 °C for 15 min in the dark. Absorbance was recorded on a plate reader (Span Autoreader 4011, New Delhi) at 450 nm after adding 0.15 mol sulfuric acid. The coefficient of variation of intra-assay was 3.5%, and that of inter-assay 9.5%.

Plasma T_3 and T_4 concentrations were measured by microwell enzyme immunoassay (EIA: magnetic solid phase) with kits (Syntron Bioresearch Inc., Carlsbad, California, Catalog # 3810-96 for T_3 and Catalog # 2210-96 for T_4) and the sensitivity of this method was checked earlier (Peter et al., 2007; Peter and Rejitha, 2011). Briefly, the anti-T_4 (goat anti-mouse IgG) coated wells were treated with 50 μL standards, controls and samples. The wells were then incubated at 37 °C (1 h) after adding T_4-HRP conjugate. After