J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

Effects of cadmium on skeletal muscles and swimming performance of *Danio rerio*

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KEY WORDS: U_{crit}, nitrogen excretion, glycogen distribution, muscle fibers resorbtion

Abstract

This study describes the physiological and cytological effects of an environmentally realistic concentration of cadmium on muscles organization and swimming performance of Danio rerio. Results indicate that the ion reduces the U_{crit} and that this effect is probably related to the resorbtion of myofibrils observed in red and white muscles.

Introduction

Cadmium (Cd) is a widespread contaminant of freshwater ecosystems that is easily uptaken by fish in which exerts highly toxic effects on gills, kidney and liver [1-3]. Cd induces significant alterations also in the muscles [3]; the consequences on swimming performance however have received limited attention. For this reason we treated adults of *Danio rerio* with an environmentally realistic concentration of Cd and determined the effects on the U_{crit}, the highest sustainable swimming speed achievable by a fish. In parallel muscles morphology and glycogen distribution in fibers were analyzed by cytological techniques.

Materials and Methods

Adults of *D. rerio* were contaminated with $CdCl_2$ dissolved in water (0.3 mg/l; Sarno River mouth, [4] and 3 mg/l). Physiological determinations and cytological analyses were carried out 15 and 30 days after the beginning of contamination. Individual U_{crit} was determined with a swimming tunnel, following a stepwise increase in water speed until fish exhausted [5]. Each step was 1BL sec^{-1} high and lasted 3 minutes [6-7]. Waste nitrogen excretion, as an index of protein catabolism, was determined measuring ammonia in water samples using the indophenol method [8] and an Hach spectrophotometer. For cytological analyses muscles sections were stained with haematoxylin-eosin to show general morphology or with periodic acid Schiff (PAS) to highlight glycogen.

Results

The U_{crit} remains constant or slightly increases during the first 15 days of treatment and then significantly decreases in 0.3 mg/l treated animals (Fig.1 a). Ammonia excretion rate (Fig.1 b) increases significantly during the first two weeks of treatment and returns to the initial value at the end of treatment. Cd causes myofibrils disorganization and partial resorbtion. At both dosages, damages appear in red muscles (Fig.1 d) and extend to white muscles (Fig.1 e, h). At the higher dosage a stretching of the endomysial fibers is also evident (Fig.1 i). This result may be related with a disruption of muscle protein turnover and agrees with the increased ammonia excretion rate in the first half of treatment period. PAS reveals that glycogen content is significantly reduced in fibers treated with 3 mg/l (Fig.1 j-l).

Discussion

Cd contamination at realistic concentrations significantly reduces swimming performance in *D. rerio*, suggesting that it may have a potentially high ecological impact, by reducing animal fitness. The reduced performance would be correlated with the significant resorbtion of myofibrils observed in red and white muscles. This event is supported by the peak in nitrogen excretion on day 15. Unexpectedly, a weaker negative effect on swim is registered at the higher Cd concentration. This could be explained considering that the higher concentration mobilizes glycogen from fibers making thus available extra energy to maintain a high swimming performance even in presence of cytological damages. In conclusion, cadmium at an environmentally realistic dosage has a toxic effect on muscles fibers thus significantly reducing swimming performance. Consequences at the ecological level are evident.

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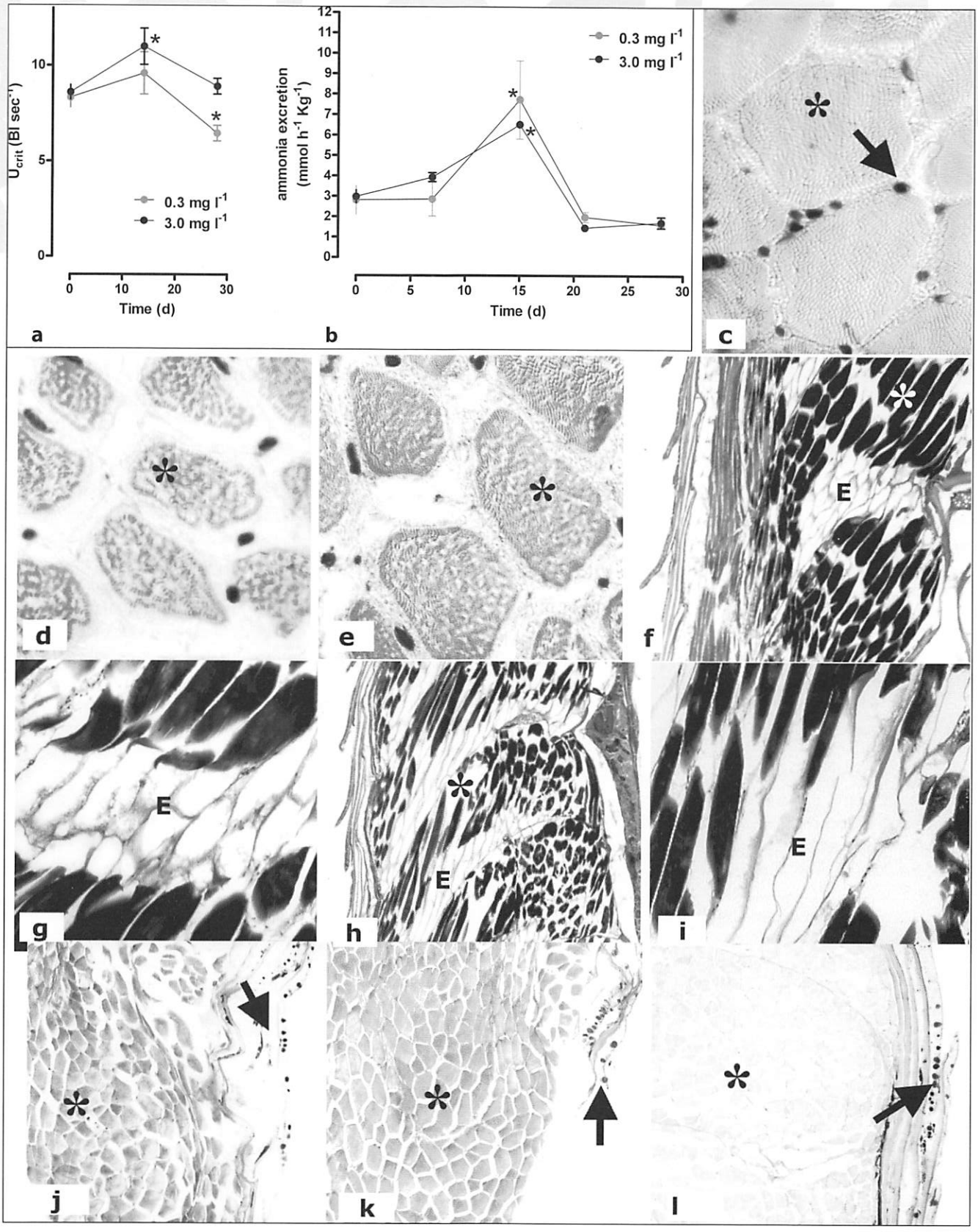

Figure 1. Effects of cadmium on U_{crit} (a) and ammonia excretion rate (b) in Danio rerio. Data are reported as means ± SE. t-Student comparison or one way ANOVA followed by Tukey post-hoc test were used as appropriate. (: significantly different, $p<0.05$). (c) untreated animal; muscle fibers with regularly arranged myofibrils (*). Fiber cells nuclei (arrow). (d) 0.3 mg/l Cd, 15 days. Red muscle fibers with disorganized myofibrils (*). (e) 0.3 mg/l Cd, 30 days. White muscle fibers with disorganized myofibrils (*). (f) untreated animal, frontal section. Muscle fibers are thick, dense and regularly packed (*); endomysial fibers (E). (g) detail. (h) 3 mg/l Cd, 15 days; frontal section. Muscle fibers are thin and disorganized (*); the endomysial fibers (E) are stretched. (i): detail. (j-l): PAS staining, cross sections. (j) untreated animal with stained fibers (*). (k): 0.3 mg/l Cd, 15 days; stained fibers (*). (l): 3 mg/l Cd, 15 days; unstained fibers (*). In figures j-l notice the intensely stained mucous cells (arrows).*

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