Effects of metabolic inhibitors on metal accumulation in juveniles of the cuttlefish Sepia officinalis

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INTRODUCTION

Previous work (Bustamante et al., 2004, Lacoue-Labarthe et al. 2009a) demonstrated that the early-life stages of cuttlefish, *Sepia officinalis*, highly accumulate the trace elements dissolved in seawater. Additionally, variations of environmental factors such as increasing partial pressure of CO_2 (pCO_2) modulate the bioaccumulation efficiencies of metals such as Ag, Cd, or Zn (Lacoue-Labarthe 2009b). One hypothesis is that cuttlefish acid-base regulation is challenged by seawater acidification, changing the gills ionic channel activities that could contribute to metal uptake in body tissues.

Based on the combined use of a unique and powerful radiotracer technique (^{110m}Ag, ^{57}Co, ^{109}Cd, ^{65}Zn; γ spectrometry) and specific metabolic inhibitors (e.g. verapamil, ouabain, 2,4 dinitrophenol, N-ethylmaleimide), we aim at identifying the ionic channels or metabolic processes involved in metal uptake.

METHOD

Sixty juveniles of the cuttlefish, *Sepia officin*alis, (2.7 ± 1.1 g) were collected in July in the Arcachon Bay and transported to the IAEA-EL premises. They were then randomly distributed in 6 groups of 10 individuals each. Juveniles were individually pre-exposed for 15 min to verapamil (10 µM), or ouabain (20 µM), 2,4 dinitrophenol (10 µM), N-ethylmaleimide (6 µM) and to control conditions (without inhibitors; n=9). They were then exposed for one hour to dissolved radiotracers with metabolic inhibitors to delineate the net metal uptake rate (k_u; min^{-1}), assuming that on this short period of time, the elimination processes are not set up and did not affected the metal bioaccumulation. Juveniles from one group (n=17) were sacrificed to assess the bioaccumulation due to adsorption of metals onto external body surface.

RESULTS

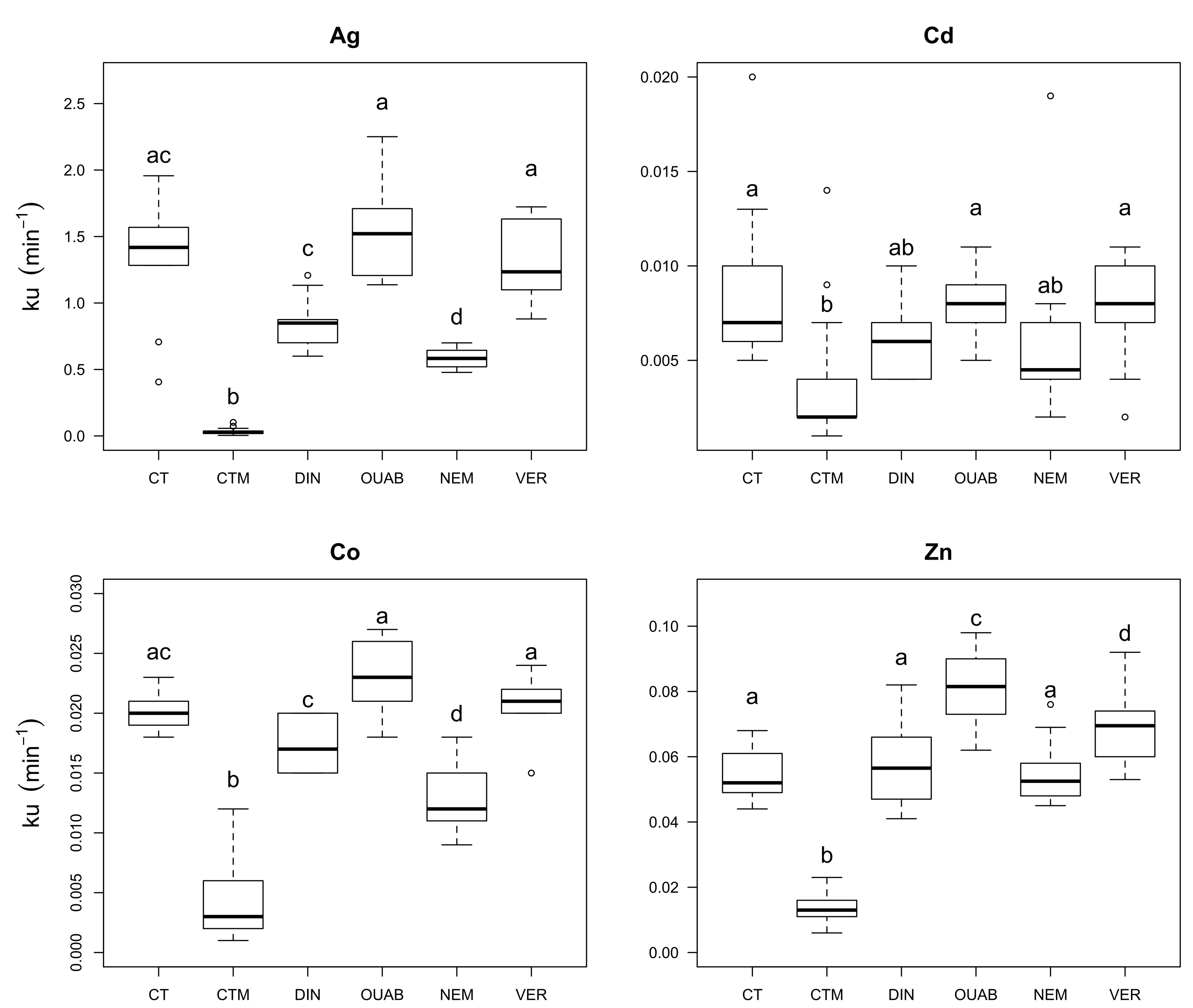

Figure. Uptake rate (k_u : min^{-1}) of ^{110m}Ag, ^{109}Cd, ^{57}Co and ^{65}Zn in juveniles exposed to dissolved radiotracers for one hour and to ionic channels and metabolic blockers; CT : control, CTM : sacrificed control, DIN : 2,4 dinitrophenol, OUAB : ouabain, NEM : N-ethylmalmeide, VER : verapamil. Different letters denote statistical differences amongk_u values (Kruskall-Wallis test, $\alpha < 0.05$)

First, comparison of the k_u measured in control and sacrificed organisms revealed that the adsorption of metal on the body surface is limited compared to real absorption in tissue. Thus, adsorption accounts for 3, 22, and 26% of the total bioaccumulation observed for ^{110m}Ag, ^{57}Co, and ^{65}Zn, respectively. Noteworthy, almost the half of ^{109}Cd (42%) activities counted in juveniles is attributed to adsorption processes.

Secondly, the specific inhibitors of the Na^+/K^+/ATPase and of calcium channels, i.e. the ouabain and the verapamil, respectively, did not lower the net uptake rates of Ag, Cd, and Co, suggesting that this ionic channels are not directly involved in these element absorption.

The influx of Ag and Co was reduced in juveniles exposed to N-ethylmaleimide, known to bind cysteine rich proteins or compounds. This inhibition suggests that the Ag and Co uptake is facilitated by mediating carrier-protein (such as glutathione).

Finally, although not significant (Kruskall-Wallis test; p = 0.08), the decrease of the Ag k_u by 2,4 dinitrophenol (which uncouples oxidative phosphorylation) implies that this influx depends on the ATP-consuming processes.

CONCLUSION. This study investigated the bioaccumulation mechanisms of dissolved metals in juvenile cuttlefish by applying *in vivo* metabolic and ionic channel blockers. Results showed that processes differ among Ag, Cd, Co and Zn and that the main machinery of element uptake mainly involves co-transporter proteins or compounds instead of ionic channels. Ag influx seems to be energy dependant. In this context, increase of Ag bioaccumulation observed when seawater pCO_2 is increasing would be linked to an increase of energy production allocated to the organism physiological responses to hypercarbia.

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