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Cadmium and Calcium Uptake in the Mollusc *Donax rugosus* and Effect of a Calcium Channel Blocker

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Donax rugosus, **a common bivalve mollusc in the coastal waters of Mauritania, has been studied for trace metal concentrations as a function of sampling site (from South of Mauritania to the North of this country) and of season (Sidoumou 1991; Sidoumou *et al.* 1992).**

In this paper, the uptake of cadmium was experimentally studied in the different organs of *D. rugosus.* Since metals such as cadmium, copper and mercury may alter calcium homeostasis (Allemand *et al.* 1989; Viarengo *et al.* 1988; Gnassia-Barelli *et al.* 1995), calcium uptake was also studied in the animals treated with cadmium. Since calcium is taken up through specific channels, it appears that metals inhibit Ca uptake by interacting with these channels in the plasma membrane. Cadmium and calcium have very similar atomic radii (Jacobson and Turner 1980), thus cadmium may be taken up through the calcium channels, particularly through voltage-dependent channels (Hinkle *et al.* 1987) . The uptake of cadmium and calcium by *D. rugosus* was therefore also studied in the presence of the calcium channel blocker verapamil.

MATERIALS AND METHODS

D. rugosus **was collected at depths between 0 and 2 m on the Mauritania coast, 15 kms north of Nouakchott. Animals were transported to the laboratory and placed in plastic tanks filled with natural, aerated seawater (S = 36°/°°, T = 21°C ± l°C) under a light–dark regime (12 hr: 12 hr). After 3 days of acclimation, cadmium-uptake experiments were carried out at two cadmium concentrations (20 and 150 µg/L) . Clams were exposed for 48 and 85 hr. The uptake of cadmium was also studied on animals previously treated with verapamil (at 100 µM, treatment 1 hr before adding cadmium) , an organic inhibitor of calcium channels.**

During exposure, the bivalves were not fed, the sea–water was renewed after 48 hr and the same treatment was

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applied (1 hr with verapamil in the case of pre-exposure, followed by addition of Cd). Control animals were held in the same manner. Consequently, there were two types of controls: those pre-exposed with verapamil and those not pre–exposed. Gills, visceral mass and the remainder of the soft tissues (i.e. , mantle, muscles and gonads) were dissected out. Organs were carefully rinsed with a buffer (10 mM Tris(hydroxymethylaminomethane) –HCl and 1 M glycine; pH = 7.8). Rinsing organs with this solution removed surface-bound Ca. In all cases, three experimental samples consisting of ten specimens each were taken from both the control and exposed animals. Experiments were carried out in triplicate.

Samples were dried to constant weight (50°C). Digestion of tissue samples was performed in a microwave oven (CEM-MDS81D) as follows. First, samples were placed in high-pressure vessels and concentrated nitric acid (65%) (Merck Suprapur) was added. The digestion procedure then followed 4 steps: microwaving at 240 W for 4 min; cooling for 10 min, microwaving again at 180 W for 4 min; and finally at 220 W for 5 min. Metal analyses were carried out in the Laboratory of Marine Toxicology (Faculty of Medicine, Nice) . Metal determinations were carried out by flame spectrometry for total calcium and by flame or furnace (for control samples) atomic absorption spectrophotometry (GBC 904 AA equipped with GF 3000) for cadmium. Deuterium background correction was used. Standard reference material (Lobster Hepatopancreas Reference Material for Trace Metals, TORT 2 provided by the National Research Council of Canada) was analyzed; the results compared well with certified values (26.3 ± 0.5 µg Cd/g dry wt, n = 5, versus the certified values of 26.7 ± 0.6 µg Cd/g) . The level of detection was 0.05 µg Cd/g dry weight) for flameless analyses of cadmium.

Data were tested for homogeneity of variance and for normal distribution. The following statistical treatment of the results consisted of two–way analysis of variance (ANOVA) , which allowed the evaluation of Cd concentration and verapamil pre–exposure on metal (Ca and Cd) concentration of the samples . Pairwise comparisons were made to determine which values differed significantly when a significant overall ANOVA was found.

RESULTS AND DISCUSSION

Figure 1 represents the accumulation of cadmium in ***D.rugosus*** in specific organs as a function of exposure time (48 and 85 hr) and of Cd concentration (20 µg/L and 150 µg Cd/L) . Cadmium uptake increased as a function of Cd concentration in the medium and exposure time.

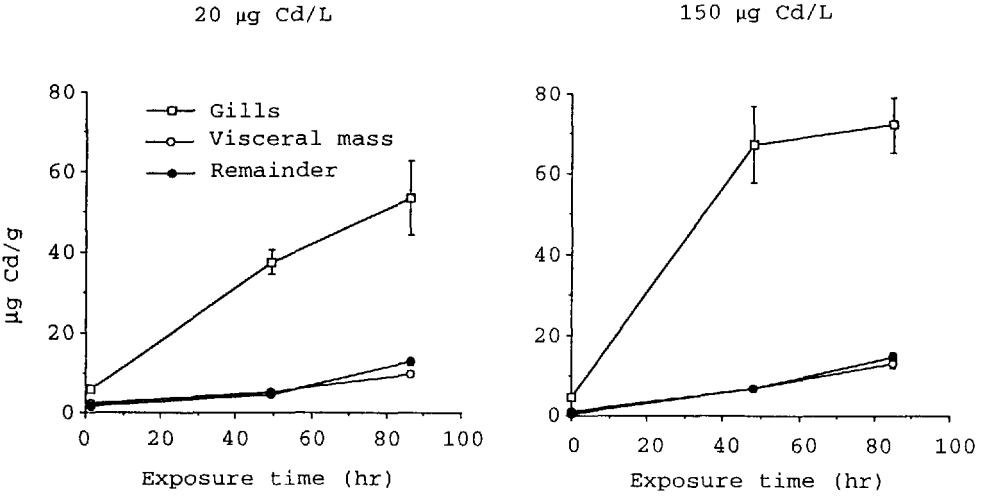

Figure 1. Accumulation of cadmium in *Donax rugosus* as a function of exposure time and of Cd concentration (20 and 150 µg Cd/L) in the medium (s.d. shown).

In all cases, gills accumulated cadmium to a much greater extent than the other organs. A decreasing range of accumulation was noted as follows: gills > visceral mass = remainder. As experiments were performed in natural sea water (without any supplement of food), the accumulation occurred by a direct mechanism of uptake where gills play a leading part. Regoli *et al.* (1991) reported a similar preferential metal (Cd, Cu and Mn) accumulation in gills of the wedge shell, *Donacilla cornea,* treated with these metals; *D. cornea* is considered to be a close relative of *D.rugosus.* At 150 µg Cd/L, saturation as a function of time appeared in the uptake of Cd by gills, all binding sites for Cd may be occupied, cadmium may then be redistributed to the other organs.

Previous work (Sidoumou 1991 ; Sidoumou *et al.* 1992) showed that cadmium uptake by *D. rugosus* linearly increased as a function of Cd concentration in the medium (from 10 µg Cd/L to 500 µg Cd/L) . According to Viarengo (1989), this phenomenon indicates that a diffusion process is involved in cadmium uptake. Using an isolated gill preparation of *Mytilus edulis,* Carpene and George (1981) reported that cadmium uptake also occurred by diffusion and that uptake was facilitated by intracellular binding and sequestration. In a previous work, Sidoumou (1991) reported that *D. rugosus* Cd levels remained unchanged following a detoxication period of 8 d, suggesting that Cd was bound firmly and sequestered in the animal by metallothioneins.

Total calcium concentration in the gills of control animals was decreased by ca 20% by verapamil after 48 and 85 hr of exposure. Comparisons (t-tests) between animals pre-exposed and not pre-exposed (after 48 hr, Fig 2 and after 85 hr, Fig 3) were significant at $p<0.05$. It must be noted that the calcium channel blocker verapamil (Vp) did not cause apparent toxicity to *D. rugosus.* Verapamil is known to block a class of calcium channels (Guerrero & Martin 1984) . The fact that verapamil decreased total calcium concentration in gills of *D. rugosus* may imply that calcium channels are present in this organ.

ANOVA was performed on results obtained after 48 hr of Cd exposure on calcium concentrations in the specific organs of *D. rugosus.* The only significant effect was that of cadmium concentration in the medium ($P<0.05$) on calcium content of gills. ANOVA, performed on calcium concentrations after 85 hr of Cd exposure, showed a significant effect of cadmium on calcium concentration in gills ($p<0.05$) and in visceral mass ($p<0.05$) and an effect of verapamil on calcium concentration in gills ($p<0.05$) .

Results concerning gills after 48-hr Cd exposure are shown in Fig 2 (left part) . A decrease in calcium concentration was observed at 150 µg Cd/L (referred to as -Vp) compared to controls (t-test significant at $p<0.01$). No significant effect was noted when animals were pre-exposed with verapamil (referred to as +Vp).

Figure 3 shows the calcium concentration in specific organs after 85 hr of Cd-exposure. A significant increase in calcium concentration was found in gills of animals exposed to 20 µg Cd/L for 85 hr (11.05 ± 3.5 µg/g compared to controls 3.96 ± 0.51 µg/g, significance of t-test shown in Fig. 3) . The same increase was found for visceral mass (t-test significant at $p<0.01$) and for the remainder of the tissues ($p<0.05$) .

The increase in calcium concentration in animals exposed to 20 µg Cd/L was not found when they were pre-exposed with verapamil (Fig 3) . Exposure of animals with 150 µg Cd/L (pre-exposed or not with Vp) did not change calcium concentration compared to controls in specific organs.

ANOVA performed on cadmium concentrations found after 48 and 85 hr of exposure in specific organs of *D.rugosus* demonstrated that the effect of cadmium concentration in the medium on cadmium concentrations was significant at $p<0.0001$. ANOVA showed that verapamil had an effect on cadmium concentration in the gills ($p<0.05$) after 48 hr, whereas after 85 hr this effect was significant in the visceral mass ($p<0.05$) and remainder ($p<0.001$).

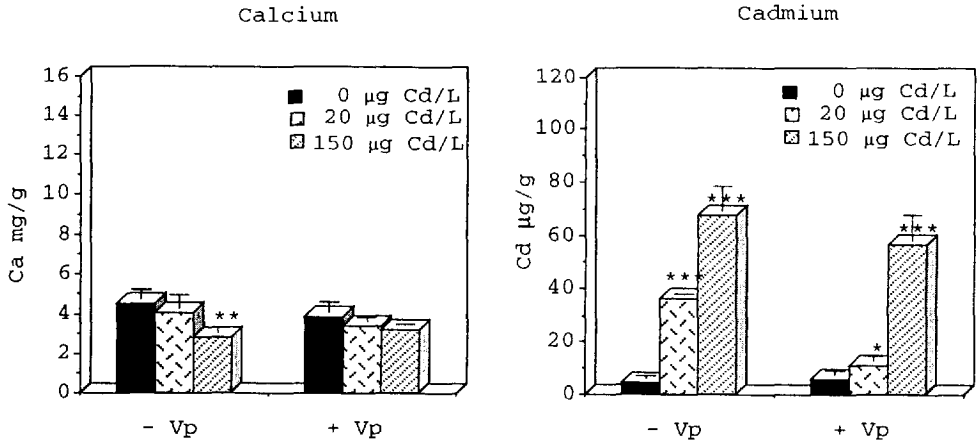

Figure 2. Ca and Cd concentrations (mean values ± 1 s. d.) in gills of *Donax rugosus* after 48 hr of Cd treatment (20 and 150 µg Cd/L). The pre-exposure with verapamil is shown by +Vp; the absence of pre-exposure by -Vp. t-tests were performed between cadmium-treated animals and appropriate controls (*p<0.05; **p<0.0l; ***p<0.00l).

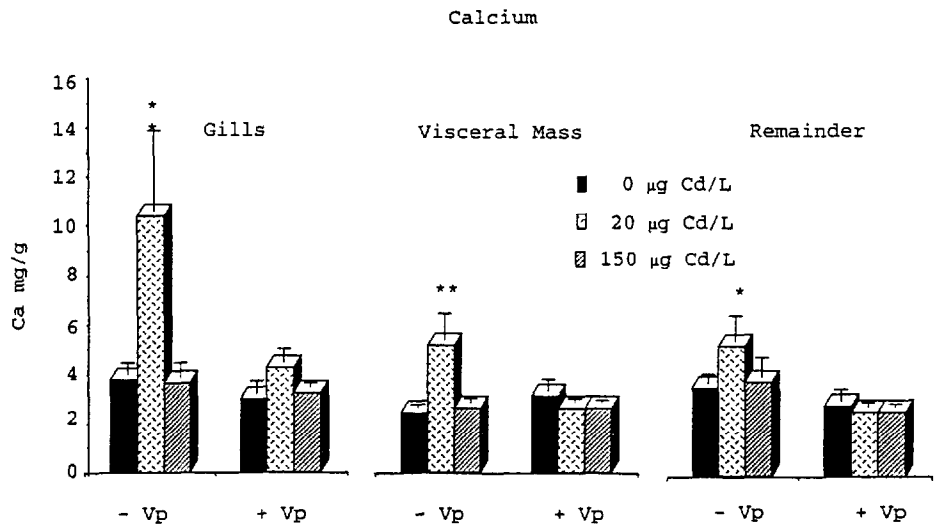

Figure 3. Ca concentrations (mean values ± 1 s.d.) in specific organs of *Donax rugosus* after 85 hr of Cd treatment (20 and 150 µg Cd/L) . The pre-exposure with verapamil is shown by +Vp, the absence of pre-exposure by -Vp. t-tests were performed between cadmium-treated animals and appropriate controls (*p<0.05; **p<0.0l; ***p<0.00l).

Results concerning cadmium concentration in gills, after 48 hr of Cd exposure, are shown in Figure 2 (right part) Cadmium in gills decreased when animals were pre-exposed to verapamil (t-test -Vp/+Vp significant at $p<0.001$ at 20 µg Cd/L; n.s. at 150 µg Cd/L).

Cadmium concentrations in the different organs after 85 hr of Cd exposure are shown in Table 1.

Table 1. Cadmium concentrations in specific organs of *Donax rugosus* after 85 h of treatment.

µg Cd/L added to the medium	Gills µg Cd/g	Visceral Mass µg Cd/g	Remainder µg Cd/g
0	6.2 ± 1.8	1.6 ± 1.1	0.3 ± 0.1
0+Vp	5.5 ± 2.0	1.8 ± 0.8	0.6 ± 0.3
20	52.1 ± 9.3	8.2 ± 0.6	11.4 ± 0.7
20+Vp	37.3 ± 2.7***	5.5 ± 0.8*	7.6 ± 0.4**
150	72.1 ± 6.8	13.3 ± 1.4	14.7 ± 0.9
150+Vp	74.2 ± 10.3 n.s	10.7 ± 0.5*	11.3 ± 0.6**

Mean values ± 1 s.d. Pre-treatment with verapamil is shown by + Vp (t–tests were performed between 20 µg Cd/L and 20 µg Cd/L + Vp and between 150 µg Cd/L and 150 µg Cd/L + Vp) t-tests significant at *p<0.05; **p<0.0l; ***p<0.001

After 85 hr of exposure (Table 1), verapamil generally decreased cadmium concentration of treated animals (20 and 150 µg Cd/L) in gills, visceral mass and the remainder (significance of t-tests -Vp/+Vp shown in Table 1). Nevertheless, verapamil had no effect on cadmium uptake by gills at 150 µg Cd/L. As mentioned above, gills had already reached saturation at this exposure time and this cadmium concentration, when animals were treated by Cd alone.

Results presented in this paper demonstrate that cadmium had an effect upon calcium homeostasis (either by decreasing calcium concentration or by increasing this concentration) in specific organs, particularly gills, of *D. rugosus.* Cadmium, introduced into the medium at 150 µg Cd/L for 48 hr, significantly decreased total calcium concentration in gills, whereas at 20 µg Cd/L and for a longer exposure time 85 hr, calcium concentration increased in gills, visceral mass and the remainder of *D. rugosus*. Alteration of calcium homeostasis by cadmium seemed, therefore, to depend on cadmium concentration and on exposure time. Sauer and Watabe (1988) reported that cadmium inhibited calcium uptake by *Fundulus heteroclitus* gills, and the authors suggested that Cd may have affected Ca uptake by competing with Ca2+ ions for the same binding sites. On

the contrary, increased calcium uptake was observed in the gills of the mussel *Mytilus galloprovincialis* treated with copper (Viarengo *et al.* 1988) and in all the organs of the clam *Ruditapes decussatus* (Gnassia-Barelli *et al.* 1995) treated with copper. *Donacilla cornea* exposed to cadmium or copper showed an increase in calcium concentration in whole soft parts and in the digestive gland (Regoli *et al.* 1991). According to Viarengo (1989), heavy metals in invertebrates may alter the plasma membrane Ca-extruding systems. This would result in a decreased capacity for pumping Ca2+ out of the cell and, therefore, in an enhanced compartmentation of Ca in the cytoplasm in an attempt to restore low physiological concentration of free cytosolic Ca2+. *D. rugosus* appeared to counter–act the toxic cadmium effect on calcium homeostasis by restoring the initial calcium concentration.

Calcium channels seem to be present in the gills of *D. rugosus.* As cadmium concentrations decreased in gills of animals pre–exposed with verapamil, cadmium may have entered through calcium channels. Since the decrease of cadmium or calcium concentrations never reached 100% compared to appropriate controls, cadmium and calcium could use other pathways than calcium channels to enter. Roesijadi and Unger (1993) demonstrated that in the eastern oyster *Crassostrea virginica* cadmium uptake by excised gills occurred through calcium channels. Using calcium channel blockers such as nifedipine, diltiazem and verapamil, the authors found that these reagents inhibited calcium uptake to a much lesser extent in comparison with cadmium and hypothesized that calcium enters by other ways than calcium channels. Consequently, cadmium uses only a subset of pathways available for calcium.

The results presented here indicated that cadmium may be taken up in *D. rugosus* via calcium channels. Nevertheless, cadmium and calcium uptake in the presence of verapamil were not totally inhibited, these metals may use other pathways (such as diffusion in the case of cadmium) .

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