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HEART RATE CHANGES OF THE MEDITERRANEAN MUSSEL (*MYTILLUS GALLOPOVINCIALIS* L.) INDUCED BY CADMIUM

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ABSTRACT

The influence of different doses of cadmium chloride on the cardiac system of the Mediterranean mussel (Mytilus galloprovincialis L.) has been tested. Non-invasive laser fiber optic photoplethismograph (LFOP) was used for simultaneous registration of the heart rate of eight sea shells. Three different concentrations of cadmium chloride: 4, 40 and 100μM/l were applied. There was no reaction in the application of the lowest dose; however the two other indicated significant reversible reaction of the heart rate. Obtained results indicate prolonged heart rate recovery time of the Mediterranean mussel after stress exposure with an increase in concentration of toxicant.

Key words: cadmium, heart rate changes, the Mediterranean mussel

INTRODUCTION

Cadmium (Cd) is a major toxic metal and represents an increasing risk for cancer as a pollutant of the environment at large (Alessandria *et al*., 2012) and also affects genome stability by inducing the generation of reactive oxygen species in cells (Kolarevic *et al*., 2013). Cadmium causes lipid peroxidation, damage to DNA, depletion of sulfhydryls, initiation of expression of heat shock proteins and methallothioneins, and induces

alteration of calcium homeostasis by competing with calcium at the Ca2+ binding sites (Molnár *et al*., 2004).

The Mediterranean mussels (*Mytilus galloprovincialis L.*) were used as test organisms since they were found to be an effective indicator of different stress conditions in the aquatic environment (Maanan, 2007, Mochino *et al*., 2011).

In this work, the influence of different doses of cadmium chloride was tested on the cardiac system of the Mediterranean mussel. Concentrations of cadmium chloride were selected based on published data on freshwater mussel *Dreissena polymorpha* (Juhel *et al*., 2007). The heart rate measurements were conducted by non-invasive fiber optic method for the registration and analysis of cardiac activity of benthic invertebrates as published by (Kholodkevich *et al*., 2008).

The goal of the study was to present the heart rate changes and to calculate mean recovery time within three groups of tested mussels after two hours treatment with three different concentrations of cadmium chloride (4, 40 and 100μM/l).

MATERIALS AND METHODS

The original laser fiber optic photoplethismograph (LFOP) was used for simultaneous registration of the heart rate of 8 individuals of Mediterranean mussel (*M.galloprovincialis* L.). The system contains an aquarium with mussels, a 8-channel LFOP unit, each channel containing an infra red light source and a receiver (placed in the channel casing), fiber optic cables for transmitting and receiving the optical signal, analog to digital signal converter and a personal computer (Fig. 1).

The Mediterranean mussels were sampled from the mussel farm in Dobrota in the vicinity of the Institute for Marine Biology, Kotor. The length of sea shells was 4 – 6 cm.

Mussels were placed in an aquarium with clean salt water, while air and water temperature was 20±1ºC. After the attachment of fiber-optic sensors to their valves by non-invasive epoxy adhesive, the animals were left in the aquarium for 12 hours with 10 liters of seawater to adapt to the laboratory conditions.

Figure 1: System for the registration of cardiac activity of aquatic invertebrates

Sensors are placed in a small casings and every single sensor contains output of transmitting and input of receiving optical fiber. Infrared light beam, originated in LFOP, illuminates the heart area of animal by transmitting fiber while receiving terminal receives the reflected light modulated by heart contractions. The information about circadian activity in the shape of analog signal is transmitted to the LFOP where is amplified,

filtrated and converted to a digital form by analog to digital converter. Digital signal is sent to personal computer via an USB port.

Three different concentrations of cadmium chloride solution: 4, 40 and 100 μM/l were applied as functional loadings. After two hours of exposure to the toxicant it was washed out and replaced with clean seawater. The recovery period of an organism after such a stress was calculated as the time needed for the restoration of HR values before the experimental changes (Martinović *et al*., 2013).

VarPulse® original software with variation pulsometry (VP) method was used to study the distribution of cardiac intervals and analyze relationships between its shape and the functioning of the cardiac system (Kholodkevich *et al*, 2008).

RESULTS AND DISCUSSION

There was no change in cardiac activity at mussels exposed to the lowest concentration of cadmium chloride (4 μM). The initial level of the heart rate was stabile throughout the time of two hours exposure, in spite of the presence of cadmium chloride.

Fig. 2 demonstrates the mean values of the heart rate within a group of 8 mussels upon of the application of 40 μM cadmium. After the latent period of 2 minutes, sharp decrease of heart rate values was the consequence of stress reaction of animal to the toxicant. A behavioural reaction of mussels was also observed in the form of valves closure. It is considered that such type of response of the cardiac system can be due to a decrease of oxygen consumption at the period when the mussel valves are closed (Kholodkevich *et al*., 2009). The functional loading was maintained for two hours and then cadmium chloride solution was washed out from the system and replaced with pure seawater. The valves remained closed a long

time after cadmium washout. The mean recovery time for 7 individual sea shells was 5 hours and 18 minutes (one of eight tested mussels did not survive).

The highest concentration of cadmium chloride (100 μM) caused a fast reaction in the form of a short increase in HR values and then fell sharply to about 50% of the initial value (Fig. 3). The duration of the functional loading was 2 hours. After washout, an increase and instability of HR was observed for a prolonged time. As the consequence of stress exposure, mussel valves were closed from the moment of stimulus application. Only one individual sea shell did not close its valves when the toxicant was absorbed in at a larger scale which caused death of this animal. The mean time of recovery for 5 individual mussels was 10 hours and 37 minutes.

In our opinion, such a long recovery time to stress after cadmium removal from the system can be caused by blocking of the voltage – sensitive calcium channels which leads to interruption of the neuromuscular synapse. According to this, the mussels are unable to open long time after washout which affects their heart rate.

CONCLUSIONS

Based on stress induced heart rate changes and mean time of recovery for the groups of mussels tested after 40 and 100 μM of cadmium chloride application, it can be concluded that a prolonged heart rate recovery time of mussels occurs with an increase in concentration of toxicant.

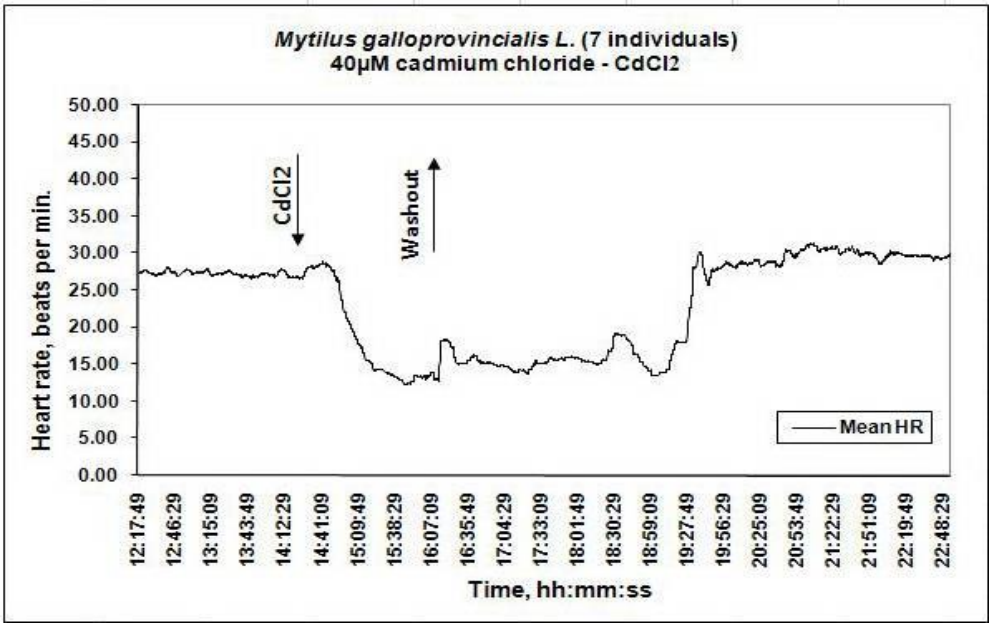

Figure 2: Change of heart contractions of the Mediterranean mussel (*M. galloprovincialis* L.) in action of 40 µM of cadmium chloride

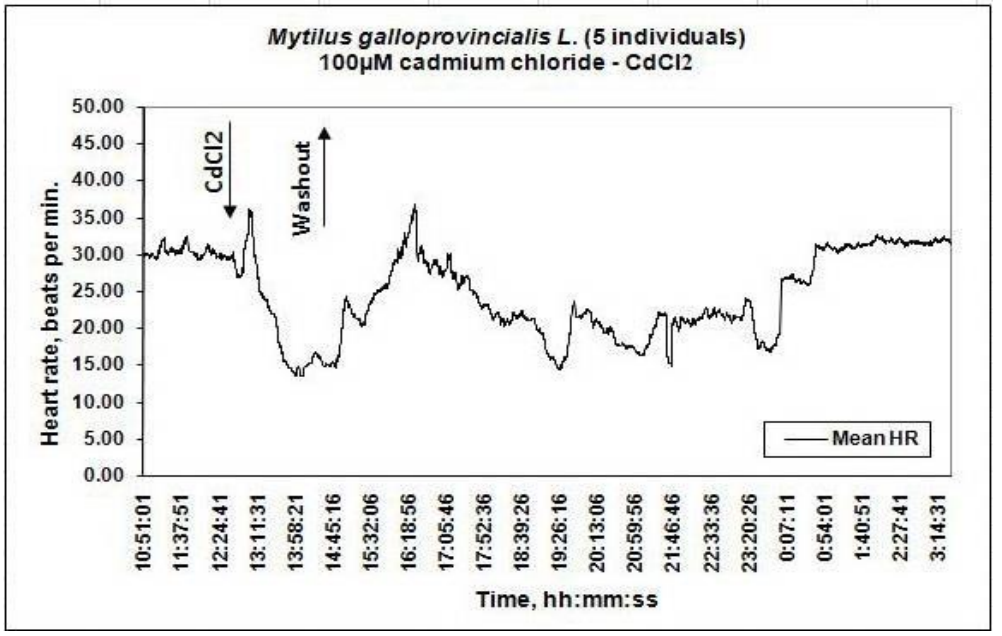

Figure 3: Change of heart contractions of the Mediterranean mussel (*M. galloprovincialis* L.) in action of 100 µM of cadmium chloride

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REFERENCES

Alessandria, I, Pennisi M., Cataudella E., Mariangela Frazzetto P., Malaguarnera M, Rampello Luigi, Rampello Liborio (2012), Neurotoxicity in cadmium-exposedworkers. Acta Medica Mediterranea, 28: 253-256.

Juhel, G., O'Halloran, J., Culloty, S.C., O'Riordan, R.M., Davenport, J., O'Brien, N.M., James , K.F., Allis, O., (2007). *In vivo* exposure to microcystins induces DNA damage in the haemocytes of the zebra mussel, Dreissena polymorpha, as measured with the comet assay. Environ. Mol. Mutagen. 48, 22-29.

Kholodkevich, S.V., Ivanov, A.V., Kurakin, A.S., etal. (2008). Real Time Biomonitoring of SurfaceWater Toxicity Level at Water Supply Stations, J. Environ. Bioindicators, vol. 3, no. 1, pp. 23–34.

Kholodkevich S. V., Kuznetsova T.V., Trusevich V. V., Kurakin A. S., Ivanov A. V. (2009). Peculiarities of valve movement and of cardiac activity of the bivalve mollusk *Mytilus galloprovincialis* at various stress actions. Journal of Evolutionary Biochemistry and Physiology , Volume 45, Issue 4, pp 524-526.

Kolarević, S., Knežević-Vukčević, J., Paunović, M., Tomović, J., Vasiljević, B., Vuković-Gačić, B.,Gačić, Z. (2013). Monitoring of DNA damage in haemocytes of freshwater mussel *Sinanodonta woodiana* sampled from the Velika Morava River in Serbia with the comet assay. Chemosphere 93(2), 243-251.

Maanan M. (2007). Biomonitoring of heavy metals using Mytilus galloprovincialis in Safi coastal waters, Morocco. Environ Toxicol. 22, (5), 525-531.

Martinović R, Kurakin, A.S., Kholodkevich, S.V., Gačić Z., Kljajić Z. (2013). Preliminary Results of Sea Water Quality Assessment Based on Physiological Biomarkers in

Part of the Boka Kotorska Bay. Water Research and Management, (ISSN 2217-5547) Volume 3, No. 1, pp. 31-34.

Molnar G., Salanki J., Kiss T., (2004). Cadmium inhibits GABA-activated ion currents by increasing intracellular calcium level in snail neurons. Brain Research 1008, 205–211.

Moschino V, Delaney E, Meneghetti F, Ros LD. (2011). Biomonitoring approach with mussel Mytilus galloprovincialis (Lmk) and clam Ruditapes philippinarum (Adams and Reeve, 1850) in the Lagoon of Venice. Environ Monit Assess. 177, (1-4):649- 663. doi: 10.1007/s10661-010-1663-5.