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Protective Action of Ions against Cadmium Toxicity to Young *Bufo arenarum* Tadpoles[1]

Claudia V. Muiño,[2] Lucrecia Ferrari,[2,3] and Alfredo Salibián[2–4]

[2]Laboratory of Ecophysiology, (Ecology Section, Biology Division), Department of Basic Sciences, National University of Lujan, P.O. Box 221, (6700) Lujan, Argentina; [3]CIC-Buenos Aires Province, Argentina, and [4]Faculty of Natural Sciences, National University of La Plata, (1900) La Plata, Argentina

Several studies have shown that a large number of amphibian species are capable of tolerating a wide range of external osmolarities (Bentley 1971; Duellman and Trueb 1986). However, the mechanisms which respond to variations in the osmotic environment are different in larvae and adults. They are also dependent on the way that the osmotic pressure is reached, i.e., through substances which behave as ionic or no-ionic ones in solution (Balinsky 1981).

The eurihalinity of tadpoles was found in species of both Urodela and Anura. The tolerated concentration rate was wide, from distilled water to several hundreds of mOsm. In the particular case of Bufo arenarum, we have demonstrated that its young tadpoles are able to survive when immersed in distilled water, NaCl and mannitol solutions of considerably higher osmolarities than its natural environment (Ferrari and Salibián 1987; Ferrari et al 1988), suggesting the existence of adaptive physiological mechanisms.

We evaluated the impact of Cd(II) on those mechanisms in the present study. Cadmium is a contaminant widely distributed in freshwater ecosystems (Ravera 1984) and known as an element that interferes in epithelial ionic permeability processes (Hayashi et al. 1977; Hillyard et al. 1979; Takada and Hayashi 1980). We hypothesized that as a consequence of those properties, Cd(II) may alter the already observed ability of Bufo arenarum tadpoles to overcome important changes in the physicochemical characteristics of their incubation media. We evaluated the effect of a sublethal concentration of this metal on the water balance of the animals

Send reprint requests to Claudia V. Muiño at the above address.

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immersing them in different solutions. Evidence is presented that inorganic ions have shown a protective action to the toxic effects of Cd(II).

MATERIALS AND METHODS

Ovulation of Bufo arenarum females was induced by injection of a suspension of homologous hypophysis into the caelomic cavity (Pisanó 1956). Oocytes were fertilized in vitro with a sperm suspension made in Holtfreter 10% solution. The composition of the stock Holtfreter solution was as follows (in g/L): NaCl 3.50; KCl 0.05; $CaCl_2$ 0.10 and $NaCO_3H$ 0.02 (Hamburger 1969). Embryos were kept at room temperature until they reached the first larval stages (stages 26/27) (Echeverría and Fiorito de López 1981). Larvae were then transferred to artificial pond water (APW) the composition of which was (in g/L): NaCl 7.60; KCl 0.75; $CaCl_2$ 8.90 and $NaCO_3H$ 1.70 (Alvarado and Johnson 1966). Tadpoles were fed every other day with pulverized fish food containing 35% protein.

All tests were conducted with animals acclimated 48 hr before the experiments began in a Ghilon TC 120 incubation chamber at constant temperature (20 $\pm$ 1.0°C) and photoperiod (12/12 hr) and remained under the same conditions throughout the experiments. For the LC50 determination the rate of one animal per 10 mL of solution was kept constant (APHA 1975); in water balance experiments the rate was one tadpole per 4 mL.

In order to establish the LC50, we assayed the effect of the following concentrations of Cd(II): 0.5, 1.0, 2.0 and 4.0 mg/L, under semistatic conditions. These solutions were freshly prepared every day dissolving appropiate amounts of a stock $CdCl_2$ solution (analytical grade) in APW. Animals in APW (5 mOsm) were considered as controls. The number of animals in each glass container was 10 for each concentration run in quadruplicate (n=40). During the 96 hr test, all solutions were renewed daily, dead animals removed and their number recorded. A specimen was considered dead when its heartbeat had stopped and/or it showed no response to gentle prodding with a glass rod. During the period of exposure to different concentrations of Cd(II), the behavioral responses and somatic alterations of the tadpoles were also recorded. The LC50 values were estimated using a probit analysis program (Finney 1971; Herrera and Mavrich 1981).

For water balance experiments, we chose, from the LC50 measurements, the concentration of 1.0 mg/L of

Cd(II) as a sublethal dose. Animals of the same age were exposed in duplicate in groups of 120 tadpoles per concentration (n=240) to the following solutions containing 1.0 mg/L of Cd(II): a) deionized water (DW; 0 mOsm) pH=5.3; b) APW (5 mOsm) pH=6.7; c) APW (30 mOsm) pH=6.8; d) sodium chloride (NaCl 141 mOsm) pH=6.8; e) mannitol (MAN 141 mOsm) pH=5.4; control tests were run simultaneously, incubating tadpoles in the same solutions without Cd(II). Solutions were changed daily. Sampling was done every 24 hr during 4 days; at each time and experimental condition, 8 samples (consisting of 4 animals each) were taken. In these samples, wet weight (ww) was determined and dry weight (dw) was determined after drying at 100-105°C. From the data of ww and dw water content (wc, expressed as mg H_2O/mg dw) and humidity (H, as percentage) were calculated. Results are expressed as means $\pm$ S.E.M of eight samples with 4 larvae each. The statistical analysis was carried out by means of a two-way analysis of variance (Cappelletti 1983; Snedecor and Cochran 1969)

RESULTS AND DISCUSSION

Effects of the assayed concentrations of Cd(II) on the survival of Bufo arenarum tadpoles are presented in Figure 1. As it can be seen, at 0.5 and 1.0 mg/L of Cd(II), mortality was less than 10%, which is an acceptable value for control animals (APHA (1975); above these concentrations toxicity showed an abrupt rise.

At the test concentrations used in this study tadpoles showed the following behavioral disorders and somatic alterations: swimming in atypical position, loss of equilibrium, increased irritability, arhythmic contractions, mild axial incurvation and epithelial peeling. The intensity of these signs was proportional to the Cd(II) concentrations.

The 96-hr LC50 for Bufo arenarum tadpoles exposed to Cd(II) was 2.08 mg/L (Table 1). This value is similar to that found by Rao and Madhyastha (1987) with tadpoles of Microhyla ornata. This level was higher than the values determined for embryos of Bufo arenarum (Pérez-Coll et al.1985,1986); from these results we conclude that the susceptibility of this anuran to cadmium is greater in the early stages of their development. The signs of toxicity observed a few hours after the beginning of the experiments provide additional evidence of the sensitivity of young tadpoles of Bufo arenarum to Cd(II). Since the tadpoles were born and bred in the laboratory in Cd(II)-free solutions, their response can be

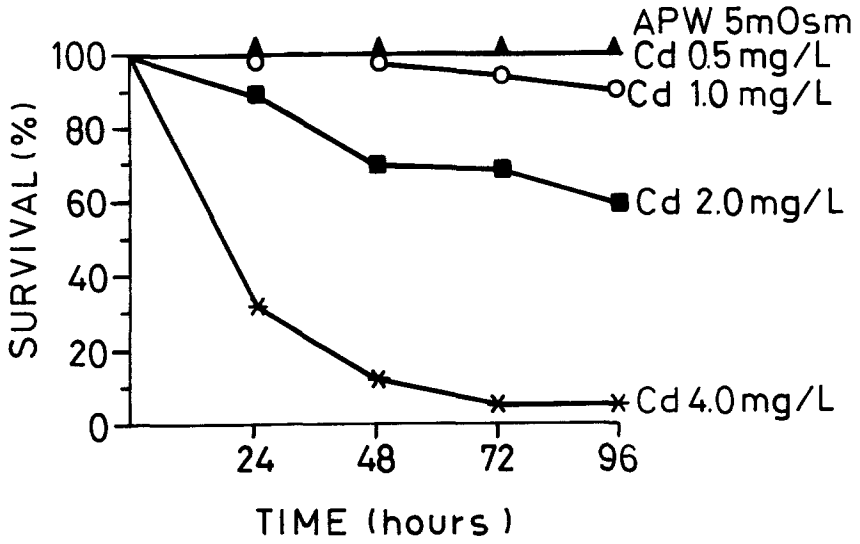

Figure 1. Survival of Bufo arenarum tadpoles (stages 26/27) exposed to different concentrations of Cd(II). (n= 40 per concentration).

Table 1. LC50 values of Cd(II) for young Bufo arenarum tadpoles at different time exposures.

Time (hr)	LC50 (mg/L)	Confidence Limits	Slope	Chi Square	Correl. Coeff.
24	3.34	2.94 - 3.38	5.85	0.05	0.99
48	2.52	2.22 - 2.86	6.01	0.51	0.99
72	2.23	1.96 - 2.54	5.59	2.20	0.98
96	2.08	1.83 - 2.40	5.13	1.44	0.99

considered devoid of any spurious effect that could be attributed to previous exposure to the metal.
In tests to determine the impact of Cd(II) on water balance of tadpoles, we found that mortality in control containers was always less than 10% . All tadpoles incubated in non-ionic solutions (MAN) or ion-free media (DW) containing 1.0 mg/L of Cd(II)were dead within 12 hr of treatment. Animals in ionic solutions (APW and NaCl) with Cd(II) had mortality comparable to that of their respective controls.

Results of experiments on water balance are shown in Table 2. There were no statistically significant differences of the various parameters in animals between control solutions of APW 5 mOsm, APW 30 mOsm

Table 2. Range of mean values (8 samples;total n of animals=32 per day) of wet weight (ww), dry weight (dw), water content (wc) and humidity (H%) of young larvae of Bufo arenarun incubated with (e) and without (c) 1.0 mg/L Cd(II).

	ww (mg)		dw (mg)		wc (mg water/mg dw)		H%	
	c	e	c	e	c	e	c	e
APW 5 mOsm	41.51 to 52.08	41.29 to 47.09	1.61 to 1.84	1.57 to 2.12	21.66 to 29.67	19.79 to 28.17	95.55 to 96.70	95.11 to 96.53
	F1 = 1.81 (ns)		F1 = 0.24 (ns)		F1 = 0.38 (ns)		F1 = 0.18 (ns)	
APW 30 mOsm	41.15 to 47.52	42.75 to 47.42	1.57 to 2.28	1.85 to 2.13	18.92 to 26.89	19.14 to 23.63	94.94 to 96.23	95.00 to 95.86
	F1 = 0.14 (ns)		F1 = 0.00 (ns)		F1 = 0.50 (ns)		F1 = 0.22 (ns)	
NaCl 141 mOsm	32.55 to 38.66	34.18 to 38.05	1.34 to 1.71	1.25 to 1.73	20.27 to 25.74	21.08 to 26.38	95.28 to 96.16	95.05 to 96.32
	F1 = 0.00 (ns)		F1 = 2.44 (ns)		F1 = 3.41 (ns)		F1 = 2.22 (ns)	

F1 = F of Snedecor between treatments
ns = not significant

and NaCl 141 mOsm and those from parallel experimental ones with 1.0 mg/L Cd(II). The experimental series of MAN and DW were not included in the statistical analysis because of its obvious results.

We demonstrated that young tadpoles of Bufo arenarum can overcome the noxious effects of the presence of 1.0 mg/L of Cd(II) when incubated in ionic solutions, whereas the same concentration of metal in ion-free or non-ionic solutions caused 100% mortality of the animals during a few hours period.

This toxicity of cadmium could be interpreted as a secondary consequence of the known inhibitory effect on epithelial ATPases. The results shown in Table 2 suggest that under our test conditions the inhibition was not associated to alterations in the water balance of the animals.

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