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Accumulation of Glutamate in Sea Anemones Exposed to Heavy Metals and Organic Amines

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Stress has been reported to accelerate protein catabolism in man and animals and as a result one can expect to observe changes in certain amino acids pools of these organisms (NICHOL and ROSEN 1963). The levels of specific free amino acids (FAA) in molluscs have been reported to fluctuate as a function of environmental stress. JEFFRIES (1972) has shown a change in the molar ratio of taurine to glycine in Mercenaria mercenaria from a polluted estuary. The change in taurine to glycine ratio was a result of both an increased concentration of taurine and a decreased concentration of glycine. In another mollusc, the clam Protothaca staminea, ROESIJADI (1979) found no change in taurine concentration but a decrease in the free glycine level following long-term exposure to chlorine in seawater. Work on Crustacea (GOULD et al. 1976) stressed with cadmium chloride has shown that the activity of the pivotal enzyme aspartate aminotransferase (AAT) in the stressed animals was increased significantly over that of the control group. GOULD and his associates interpret this as a method of potentially providing the extra energy necessary to withstand cadmium stress. AAT activity also has been found to increase in newly hatched brook trout in response to cadmium and methyl mercury stress (CHRISTENSEN 1975). In each case at least a temporary change in glutamate level as well as the levels of other FAA's would be expected to result following the increase in AAT activity. In fact, MEHRLE and BLOOMFIELD (1974) showed that following 240 days of exposure to dieldrin, the brains of brook trout had at least a doubling in the concentration of free glutamate as well as aspartate, glycine, proline, and alanine.

In the present study, the Gulf Coast sea anemone, Bunodosoma cavernata, was used as the test animal and free amino acid levels of whole animals were measured following stressed conditions. Sea anemones were chosen as the test animals since they are sessile and, due to the nature of their morphology, they have few mechanisms by which they can escape environmental stress. The animals were exposed to sublethal concentrations of the metals; mercuric chloride and cadmium chloride and the organic amines; aniline, diethanol amine (DEA) and ethylene diamine (EDA), Chloride salts of mercury and cadmium were chosen rather than other anions since chloride is the most abundant anion in seawater. These two particular metals were chosen as challenge compounds due to their high toxicity in aquatic systems. The three organic amines were chosen for their relatively high water solubility and low vapor pressure in an aqueous solution thus insuring that the toxic compound is retained in the test solution. Since organic amines are used extensively in the Gulf Coast industrial complex (HAHN 1970), there is a high probability of these compounds contaminating the marine environment.

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MATERIAL AND METHODS

Sea anemones were collected off the jetties in Galveston, Texas and were acclimated in the laboratory for at least two weeks in artificial seawater (Instant Ocean) at 26 o/oo salinity prior to experimentation. They were fed minced clams at biweekly intervals. All toxic exposures were performed at room temperature in one liter glass beakers with 500 ml of 26 o/oo seawater to which the substances were added at calculated sublethal doses. Water was changed daily and aerated throughout the exposure period.

Sublethal exposure level for each of the five test compounds were determined by first performing acute lethality assays and then reducing the quantity of the test substance to the concentration at which the anemones were able to survive one week and return to normal after the test substance was removed. The concentrations used were 1.2 ppm Hg^{++} as $HgCl_2$, 7 ppm Cd^{++} as $CdCl_2$, 50 ppm ethylene diamine, 150 ppm diethanolamine and 500 ppm aniline.

Animals were exposed either for 24 hours or 7 days under the test conditions. Following exposure to the toxic substances each anemone was frozen and lyophololyzed for dry weight determination. The dried animals were cut up and homogenized in water to which norleucine had been added as an internal standard. The quantity of norleucine added was based on the dry weight of each animal. Following homgenization, 50% cold trichloroacetic acid (TCA) was added to make a final concentration of 10% TCA to precipitate the macromolecules. After centrifugation at 15,000 x g the supernatant containing the FAA fraction was extracted in ether to remove the TCA and then lyopholyzed. The FAA lyopholyzate was diluted in 0.1 N HCl for analysis on an Amino Aminalyzer using a 2.5 hour citrate buffer system. Quantities of each amino acid were determined with a flourescence detector after reaction with orthophthaldialdehyde. Significance of differences between means was determined by t-test for independent means.

RESULTS AND DISCUSSION

Since taurine, glycine, alanine, glutamate, and aspartate make up 92% of the total FAA pool in B. cavernata only these five amino acids will be discussed in relation to responses to environmental stress. The remaining amino acids are present in such low concentrations that even if they underwent statistically significant changes in concentration only a minimal effect would be observed by the animal. The most noticible response of the anemones to heavy metal toxicity is the 50% increase in free glutamate concentration during the first 24 hours of exposure followed by a continued increased over a week until the concentration is 3 to 4 times that of the control animals (Table I). Alanine also shows a 50% increase over the 7 day duration of the experiment.

As can be seen in Table II, the reaction of B. cavernata to stress from organic amines is similar to the response to heavy metals, only more extensive. Free glutamate shows a 50 to 150% increase in the first 24 hours and by 7 days has increased 3 to 4 fold over that of the control animals. Animals show a greater increase in free alanine levels following treatment with ethylene diamine and diethanolamine than aniline, mercury, or cadmium.

TABLE I

Concentrations of free amino acids in Bunodosoma cavernata following 1 and 7 day sublethal exposures to heavy metals[x]

Amino acid	Control	Treatment $HgCl_2$ (1.2 ppm)		Treatment $CdCl_2$ (7 ppm)	
		1 day	7 days	1 day	7 days
Aspartate	6.0 (0.8)	5.4 (1.5)	6.2 (0.7)	8.7 (1.6)	6.6 (0.9)
Glutamate	6.0 (0.6)	10.0* (1.7)	21.1+ (3.3)	10.0* (1.0)	25.2+ (2.7)
Glycine	66.0 (18.0)	21.0 (14.6)	24.6 (17.1)	42.5 (13.4)	55.0 (24.4)
Alanine	6.4 (0.3)	5.7 (0.8)	10.1* (1.5)	9.3 (1.8)	9.8* (2.5)
Taurine	98.6 (10.3)	101.6 (9.8)	110.1 (8.2)	92.0 (13.9)	133.4 (16.1)

[x]The values represent the mean (± SE) of 4 animals in μmoles/g dry weight.

* Increase over control at $p < .05$

+ Increase over control at $p < .01$

TABLE II

Concentrations of free amino acids in Bunodosoma cavernata following 1 and 7 day sublethal exposures to organic amines[x]

Amino acid	Control	Treatment EDA (50 ppm)		Treatment DEA (150 ppm)		Treatment aniline (500 ppm)	
		1 day	7 days	1 day	7 days	1 day	7days
Asparate	6.0 (0.8)	13.0^{+}(0.4)	13.6*(2.8)	12.2*(1.0)	7.9 (0.9)	6.3 (1.6)	9.4*(1.8)
Glutamate	6.0 (0.6)	16.6*(0.1)	25.2^{+}(0.5)	13.5^{+}(1.7)	23.6*(5.1)	10.5*(1.1)	20.8^{+}(2.2)
Glycine	66.0 (18.0)	11.6 (1.2)	29.3 (21.8)	36.4 (19.4)	26.8 (12.5)	41.9 (17.4)	94.6 (59.7)
Alanine	6.4 (0.3)	19.4^{+}(1.2)	22.5^{+}(1.4)	11.8*(2.3)	15.8^{+}(1.2)	9.2 (1.2)	10.4^{+}(0.8)
Taurine	98.6 (10.3)	86.2 (17.7)	130.6 (7.2)	98.5 (18.4)	95.8 (18.4)	101.2 (6.3)	105.6 (9.8)

[x]The values represent the mean (± SE) of 4 animals in µmoles/g dry weight.

[*]Increase over control at $p < .05$

[+]Increase over control at $p < .01$

The most consistent and significant change observed following sublethal toxic stress is that of increased levels of free glutamate. This is in agreement with data shown by MEHRL and BLOOMFIELD (1974) in their study on ammonia detoxification mechanisms in dieldrin treated trout. They found a doubling of the brain free glutamate, which alone makes up 52-54% of the the brain FAA pool, as well as significant increases in aspartate and alanine. These changes occurred in combination with a decrease in brain glutamate dehydrogenase (GDH) activity suggesting a disruption in ammonia detoxification mechanisms in trout stressed in dieldrin. While glutamate levels were not specifically measured, GOULD et al. (1976) demonstrated in rock crabs and CHRISTENSEN (1975) showed that in newly hatched trout AAT activity increased significantly during heavy metal treatments. This increase in activity could potentially result in increased concentrations of glutamate. Thus the suggestion of increased utilization of proteins as an energy source in stressed animals (NICHOL and ROSEN 1963) combined with the increased activity of AAT and decreased activity of GDH could easily result in increased levels of free glutamate. This then suggests that free glutamate levels at least in some animals may be a much better indicator of polluted environments than either glycine levels or a glycine to taurine ratio.

While free glycine in B. cavernata generally shows a reduction in concentration in response to sublethal doses of heavy metals or organic amines, its usefulness in indicating stress responses in anemones is doubtful since there is a tremendous variation in free glycine values from animal to animal. This is apparently a seasonal response since control animals collected in the winter have an average of 12.5 $\pm$ 3.2 μmoles glycine/g dry weight compared to 22.2 $\pm$ 13.2 μmoles glycine/g dry weight for animals collected in the fall and 66.0 $\pm$ 18.0 μmoles glycine/g dryweight for animals collected during the summer months (KASSCHAU, unpublished observations). Large seasonal variations in free glycine levels have also been observed in the barnacle, Balanus balanoides (COOK et al. 1972) and the seastar, Echinaster modestus (FERGUSON 1975). The taurine levels in B. cavernata, however, remained relatively consistent throughout all the toxic exposure conditions.

Thus, for anemones it appears that not only are changes in the glycine:taurine ratio as suggested by JEFFRIES (1972) inappropriate indicators of environmental stress, but also drawing conclusions about stress based on a reduction in free glycine levels alone as suggested by ROESIJADI (1979) is invalid due to the very high variability in individual glycine concentrations. However, in this sessile group of animals with no apparent mechanisms to reduce environmental stress, increases in the concentration of free glutamate is a good metabolic indicator of a response to a number of toxic compounds. Glutamate levels are not only very consistent from animal to animal within each experimental group as is exemplified by the low standard error values, but the absolute increase in concentration under sublethal stress from a number of different compounds is very consistent and well above the baseline level.

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REFERENCES

CHRISTENSEN, G. M.: Toxicol. Appl. Pharmacol. 32, 191 (1975).

COOK, P. A., P. A. GABBOTT and A. YOUNGSON: Comp. Biochem. Physiol. 42B, 409 (1972).

FERGUSON, J. C.: Comp. Biochem. Physiol. 51A, 341 (1975).

GOULD, E., R. S. COLLIER, J. J. KAROLUS, and S. GIVENS: Bull. Environment. Contam. Toxicol. 15, 635 (1976).

HAHN, A. V.: The Petrochemical Industry. New York: McGraw-Hill Book Company 1970.

JEFFRIES, H. P.: J. Invert. Pathol. 20, 242 (1972).

MEHRLE, P. M., and R. A., BLOOMFIELD. Toxicol. Appl. Pharmacol. 27, 355 (1974).

NICHOL, C. A. and F. ROSEN: in Advances in Enzyme Regulation, Vol. 1. Editor WEBER, G., Oxford: Pergamon Press 1963.

ROESIJADI, G.: Bull Environm. Contam. Toxicol. 22, 543 (1979).