

Effect of octopamine on (Na⁺,K⁺)-ATPase activity in selected ontogenetic stages of *Macrobrachium amazonicum*

Malson Neilson de Lucena⁽¹⁾; Marcelo Rodrigues Pinto⁽²⁾; John Campbell McNamara⁽³⁾, Francisco de Assis Leone⁽²⁾

⁽¹⁾Departamento de Bioquímica, FMRP, Universidade de São Paulo, Ribeirão Preto.

⁽²⁾Departamento de Química, FFCLRP, Universidade de São Paulo, Ribeirão Preto. ⁽³⁾

Departamento de Biologia, FFCLRP, Universidade de São Paulo, Ribeirão Preto.

neilson_bio@yahoo.com.br

Introduction: Crustaceans are predominantly marine organisms. Although many have become independent of seawater, completing their entire life cycles in freshwater, others still appear to be invading this medium, as suggested by their larval developmental sequence being dependent on brackish water and by their characteristic metabolic, osmotic and ion regulatory mechanisms. Several biogenic amines have been proved to be involved in the osmoregulatory mechanisms of various decapods crustaceans. **Objective:** The effect of the octopamine on (Na⁺,K⁺)-ATPase activity in whole zoeae I and decapodid III, and in gill homogenates from juvenile and adult *M. amazonicum* was investigated. **Methods:** The effect of exogenous octopamine on (Na⁺,K⁺)-ATPase activity was examined after pre-incubation of the enzyme for 10 min at 25 °C with increasing octopamine concentrations. The gills or whole individuals were rapidly diced and homogenized in 20 mM imidazole, pH 6.8, 6 mM EDTA, 250 mM sucrose and protease inhibitor cocktail (homogenization buffer, 20 mL/g wet tissue). After centrifuging the crude extract at 20,000 × g for 35 min, at 4 °C, the supernatant was placed on crushed ice, and the *pellet* was re-suspended in an equal volume of homogenization buffer. After further centrifugation as above, the two supernatants were pooled and centrifuged at 100,000 × g for 3 h, at 4 °C. The resulting *pellet* was re-suspended in the homogenization buffer (10 mL/g wet tissue). **Results:** Total ATPase activity from decapodid III was considerably inhibited (-75%) by 100 mmol⁻¹ octopamine, which was less effective inhibitor for zoea I, juvenile and adult (-65%, -65% and -48%, respectively) enzyme. As octopamine concentration increased from 10⁻⁵ to 10⁻¹ M, total ATPase activity from zoea I, decapodid III, juvenile and adult, decreased from 180 U mg⁻¹ (1 U/mg = 1 nmol Pi⁻¹ min⁻¹ mg), 344 U mg⁻¹, 231 U mg⁻¹ and 154.1 U mg⁻¹ to less than 65 U mg⁻¹, 77 U mg⁻¹, 69 U mg⁻¹ and 79 U mg⁻¹, respectively. Ouabain insensitive ATPase activity varied little over the same octopamine concentration range, suggesting that only (Na⁺,K⁺)-ATPase activity is affected by this octopamina. The calculated K_i for octopamine for (Na⁺,K⁺)-ATPase was 60.66±1.6 mM, 38.22±3.6 mmol mM, 47.57±1.43 mM, 120.35±6.02 mM for zoeae I, decapodid III, juveniles and adults, respectively. **Conclusion:** Octopamine alters (Na⁺,K⁺)-ATPase activity of the microsomal fraction of different ontogenetic stages of selected *M. amazonicum* and the different responses disclosed suggest that it may play an important role in the invasion of freshwater by this species.

Keywords: Octopamine, *M. amazonicum*, ontogenetic stage, (Na⁺,K⁺)-ATPase inhibition, gill microsome

Financial support: FAPESP, (INCT) ADAPTA/Fundação de Amparo à Pesquisa do Estado do Amazonas, CNPq, CAPES.