Effect of Nitrite Exposure on $K^+$-Phosphatase Activity of the Gill (Na$^+$, K$^+$)-ATPase in the Amazon River Shrimp *Macrobrachium amazonicum*

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**INTRODUCTION:** The (Na$^+$, K$^+$)-ATPase transports three Na$^+$ out of and two K$^+$ into the cell cytoplasm for each molecule of ATP hydrolyzed. Nitrite is essential to life and constitutes one of the most common dissolved inorganic nitrogen ions within aquatic ecosystems. However, excessive nitrite levels can significantly affect the abundance and physiological condition of aquatic animals, including decapod crustaceans.

**Objectives:** In this study, we investigate nitrite toxicity in *M. amazonicum*, focusing specifically on the K$^+$-phosphatase activity of the gill (Na$^+$, K$^+$)-ATPase.

**MATERIAL AND METHODS:** Sodium nitrite was dissolved in aerated water at 7.5 mg L$^{-1}$ and 15 mg L$^{-1}$ final concentration and shrimps were exposed for three days. Gill K$^+$-phosphatase activity was measured continuously at 25°C, through the release of p-nitrophenolate ion. Standard conditions were: HEPES 50 mmol L$^{-1}$, pH 7.5, containing p-nitrophenylphosphate (PNPP) 10 mmol L$^{-1}$, MgCl$_2$ 7 mmol L$^{-1}$ and KCl 10 mmol L$^{-1}$, in a final volume of 1 mL. Activity was also measured under the same conditions with 3 mmol L$^{-1}$ ouabain.

**RESULTS AND DISCUSSION:** PNPP stimulated the K$^+$-phosphatase activity of the (Na$^+$, K$^+$)-ATPase according to a single saturation curve with $V_{M}= 8.35$ nmol min$^{-1}$ mg$^{-1}$, and $K_{0.5}= 1.35$ mmol L$^{-1}$. For shrimps exposed to 7.5 mg L$^{-1}$ nitrite, PNPP was hydrolyzed at a maximum rate of $V_{M}= 9.07$ nmol P$_i$ min$^{-1}$ mg$^{-1}$ with $K_{0.5}= 0.90$ mmol L$^{-1}$. Shrimps exposed to 15 mmol L$^{-1}$ nitrite, showed increased PNPP hydrolysis with a maximum rate of $V_{M}= 13.43$ nmol P$_i$ min$^{-1}$ mg$^{-1}$ and $K_{0.5}= 1.02$ mmol L$^{-1}$. Modulation of enzyme activity by magnesium (V$= 14.13$ nmol min$^{-1}$ mg$^{-1}$, and $K_{0.5}= 1.50$ mmol L$^{-1}$) and potassium ions (V$= 12.63$ nmol min$^{-1}$ mg$^{-1}$ and $K_{0.5}= 2.30$ mmol L$^{-1}$) showed positive cooperativity.

**CONCLUSION:** Increased nitrite concentration increases $V_M$, while $K_{0.5}$ remains unchanged.

**Keywords:** nitrite exposure; (Na$^+$, K$^+$)-ATPase; *Macrobrachium amazonicum*

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