Rhesus glycoproteins in dogfish shark (*Squalus acanthias*) in response to hypo-osmotic stress

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Rhesus (Rh) proteins are a new class of ammonia transporters that facilitate NH_3 diffusion. Our aim was to investigate whether the Rh proteins are expressed in dogfish tissues and whether hypo-osmotic stress alters tissue ammonia balance and Rh protein expression. We found that Rhcg was expressed in the gill and kidney and tissue ammonia levels did not change with decreased plasma osmolarity. Work is ongoing to quantify Rh protein expression.

Marine elasmobranchs osmoconform by maintaining elevated organic osmolytes, *e.g.*, urea, to counterbalance the saline environment. Ammonia is retained to form glutamine, the nitrogen-donating substrate for urea synthesis. Under hypo-osmotic conditions, urea synthesis is curtailed[1], and excess ammonia may accumulate or be excreted. Since ammonia excretion in teleost fishes is facilitated by Rh proteins[2], we tested the hypothesis that Rhcg is critical for ammonia retention or excretion in dogfish shark and expression is regulated to maintain ammonia homeostasis with changing external salinity.

Blood was collected from the caudal artery (with MDIBL IACUC approval) after 48-h exposure to dilute (70%) seawater (SW) or control (100%) SW. Plasma osmolarity and urea, ammonia, Na^+ and Cl^- concentrations were determined. Sharks were euthanized; tissues were collected for analysis of urea and ammonia concentrations and Rhcg, Na^+/H^+ exchanger (NHE2, NHE3) protein by immunoblot and immunohistochemistry.

Hypo-osmotic stress significantly reduced plasma osmolarity (Fig 1; black bars: Control, white bars: 70% SW). Tissue ammonia concentrations did not change, but plasma and muscle urea concentrations were lower (Table 1). Gill and kidney cells were immunoreactive to hagfish Rhcg antibody (generated by Appalachian State University) with a strong signal in the apical region. This is the first report of tissue localization of Rhcg expression in an elasmobranch.

Table 1. Tissue ammonia and urea concentrations (mM).

Tissue	100% Seawater	70% Seawater
Plasma: *Ammonia*	34.17±9.4	58.94±16.7
Urea	726.53±32.0	575.95±13.0 *
Kidney: *Ammonia*	531.35± 59.7	532.09±101.2
Urea	608.85±90.5	483.86±-63.3
Muscle: *Ammonia*	260.15±58.2	303.15±22.8
Urea	720.48±91.5	517.03±78.0 *

Mean±SEM (n = 5-7); *P < 0.05 *vs*. 100% SW, per *t*-test.

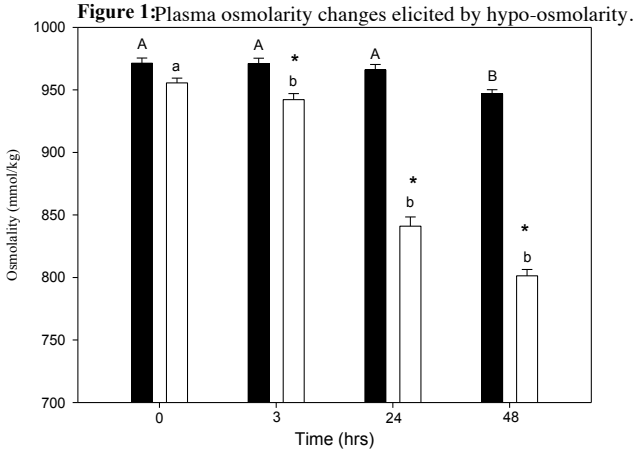

Figure 1: Plasma osmolarity changes elicited by hypo-osmolarity.

This research was supported by a MDIBL Dave Evans Visiting Faculty Fellowship to PW, an NSERC Discovery grant to PW, SC and CMW and NSF IOS 1121369 to SE.

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