EFFECT OF RECTAL GLAND REMOVAL ON Na^+,K^+-ATPase EXPRESSION IN THE GILLS AND KIDNEYS OF *RAJA ERINACEA*

Peter M. Piermarini[1], Keith P. Choe[2], and Karl J. Karnaky, Jr.[3]
[1]Department of Zoology, University of Florida, Gainesville, FL 32611
[2]Department of Biology, Georgia Southern University, Statesboro, GA 30460
[3]Department of Cell Biology and Anatomy, and the Marine Biomedical and Environmental Sciences Program, Medical University of South Carolina, Charleston, SC 29425

Several studies have shown that elasmobranchs can survive removal of their rectal gland with no, or only a slight, change of plasma NaCl concentrations (Burger, J.W. *Physiol. Zool.* 38: 191-196, 1965; Chan, D.K.O *et al. Comp. Biochem. Physiol.* 23: 185-198, 1967; Forrest, J.N. *Bull. MDIBL* 13: 41-42, 1973; Haywood, G.P. *J. Exp. Zool.* 193: 167-176, 1975; Evans, D.H. *et al. J. Exp. Biol.* 101: 295-305, 1982; Wilson, J.M. and Randall D.J. *Exp. Biol. Online* 1: A9.47, 1996). The rectal gland is considered the major site of net ion excretion in elasmobranchs, so it is surprising that removal of this gland does not cause more substantial ion regulatory disturbances. Clearly other organ(s) must be involved with net ion excretion and compensate for the loss of the rectal gland. Two potential sites of this compensatory ion excretion are the gills and kidneys.

Burger (*op. cit.*) demonstrated that *Squalus acanthias* without rectal glands had elevated renal chloride loss through increased urine flow, but he concluded that "...the kidneys can not serve as a substitute rectal gland." Wilson and Randall (*op. cit.*) measured activity of Na^+,K^+-ATPase in the gills and kidneys of *S. acanthias* without rectal glands, and did not find any difference relative to sham operated animals. Additionally, Wilson and Randall (*op. cit.*) found no difference in the numbers of branchial mitochondrion-rich cells. The goal of this study was to determine if removal of the rectal gland had an effect on the expression of Na^+,K^+-ATPase in the gills and kidneys of the little skate, *Raja erinacea*. If either of these organs is involved with compensatory ion excretion, they may be the site of up-regulated expression for this key active transport enzyme. To our knowledge, the effect of rectal gland removal has never been investigated in a skate species, therefore, it was also necessary to determine what effects removal of the gland had on plasma NaCl concentrations.

The skates (n=11) for this study were captured from Frenchman Bay and held in running sea water for the entire experiment. Rectal glands were removed from six skates (experimental), while the other five skates were sham operated (control), as described in Evans et al. (*op. cit.*). Both groups were held in the same tank. To monitor plasma ion concentrations, blood samples (0.5 ml) were taken via caudal puncture before the operation, and every other day for 10 days after the operation. Plasma was isolated and analyzed for Na^+ and Cl^- using a flame photometer and Cl^--titrator, respectively. A Mann-Whitney U-test was used to compare control and experimental plasma values on a given day. A Wilcoxon matched-pairs test was used to compare initial and final plasma values within a group. On the final day of the experiment, skates were pithed and membranes from gills and kidneys were enriched as described by Choe et al. (Bull. MDIBL 37: 38-39, 1998). Expression of Na^+,K^+-ATPase was quantified using Western blot analysis as described by Choe et al. (*op. cit.*), except the monoclonal antibody a5 was used; this antibody is specific for the 112 kDa alpha-subunit of Na^+,K^+-ATPase. The monoclonal antibody a5 developed by Dr. D.M. Fambrough was obtained from the Developmental Studies Hybridoma Bank maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242.

Initial plasma Na^+ and Cl^- concentrations in the control (C) and experimental (E) skates were not statistically different (C Na^+ = 257.4 ± 2.1 mM, E Na^+ = 258.67 ± 0.6 mM; C Cl^- = 245.5 ± 3.8 mM, E Cl^- = 252.86 ± 1.25 mM). Two days after the operation, both plasma Na^+ and Cl^- concentrations in the experimental skates were significantly greater than those in the control skates (C Na^+ = 250.30 ± 5.45 mM, E Na^+ = 263.92 ± 1.23 mM; C Cl^- = 240.17 ± 5.2 mM, E Cl^- =

253.77 ± 2.14 mM). Plasma NaCl concentrations remained elevated in the experimental skates, while NaCl in the control skates fluctuated. However, plasma NaCl concentrations in the control skates were never greater than those of the experimental skates. On the final day of the experiment, plasma Na^+ concentrations in the experimental skates were significantly greater than those in control skates (C = 270.6 ± 0.43 mM, E = 282 ± 1.69 mM), but plasma Cl^- concentrations were statistically equivalent (C = 251.47 ± 5.24 mM, E = 268.31 ± 3.91 mM). Regardless, final plasma Na^+ and Cl^- concentrations were significantly greater than initial values in the experimental skates, while the final and initial values were not statistically different in the control skates. Therefore, it appears that removal of the rectal gland causes an increase in plasma NaCl concentrations, but the plasma was still substantially hypo-ionic relative to sea water. The variability of plasma NaCl in the control skates can not be directly accounted for, but is possibly due to leakage of sea water through the surgical sutures.

Using Western blots, we tried to detect differences in expression of Na^+,K^+-ATPase in the gills and kidneys. Results of the Western blotting from gill and kidney tissue detected a band in the expected molecular weight range. The optical density of this band, however, was not significantly different between the control and experimental skates, for either tissue type (C Gill = 32.1 ± 7.2 arbitrary units, E Gill = 41.6 ± 10.3 arbitrary units; C Kidney = 74.3 ± 6.4 arbitrary units, E Kidney = 69.8 ± 3.7 arbitrary units). This suggests that removal of the rectal gland does not have a significant effect on the total expression of Na^+,K^+-ATPase in the gills and kidneys, which corroborates the findings of Wilson and Randall (*op. cit.*).

In summary, we found that removal of the skate rectal gland causes a rise in plasma NaCl concentrations, which remain well below sea water concentrations of NaCl. Regulation of this elevated plasma NaCl is not associated with an up-regulation of Na^+,K^+-ATPase in gill or kidney tissue. However, this finding should not rule out the possibility that there may be branchial and/or renal compensatory salt excretion, since there are other key proteins involved with ion excretion such as the $Na^+,K^+,2Cl^-$-cotransporter and cystic fibrosis transmembrane conductance regulator. Future research should determine if there are any changes in the expression of these transport proteins before the idea of extra-rectal gland salt excretion in elasmobranchs is dismissed.

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