Further evidence for a transporter mediated urea uptake in the rectal gland of *Squalus acanthias*

Patricio Silva[1], Anya Silva[2], Milena Silva[3] and Katherine C. Spokes[4]
[1] Department of Medicine Temple University School of Medicine, Philadelphia, PA 19140
[2] Wellesley College, Wellesley, MA 02481
[3] Phillips Academy Andover, Andover, MA 01810
[4] Department of Medicine Beth Israel Deaconess Medical Center
and Harvard Medical School, Boston, MA 02215

Cartilaginous fish such as sharks, skates, and rays prevent the water from leaving their bodies by increasing the amount of solute in their tissues and body fluids. The main solute they retain is urea. The studies reported here show that the cells of the rectal gland of the shark contribute to the retention of urea by limiting the amount of urea secreted.

The concentration of urea in the plasma of the shark is 350 mM. Isolated shark rectal glands are perfused with a similar concentration of urea. The concentration of urea in the secretion of the rectal gland is very low[1]. The osmolality of the rectal gland secretion is the same as that of the plasma[1] or perfusate. Decreasing the concentration of urea in the perfusate of isolated glands decreases the concentration of salt in the secreted fluid[3], while increasing the concentration of urea in the perfusate increases the concentration of salt in the fluid[2]. During these maneuvers, the osmolality of the secretion of the rectal gland remains isotonic with the perfusate. Because of the absence of urea from the secretion of the rectal gland the concentration of salt in the secretion almost doubles. We and others have suggested that this doubling of the concentration of salt in the secretion is the result of the virtual absence of urea from the secretion. Rectal gland cells contain urea at the same concentration present in plasma or perfusate[4]. We have suggested that urea enters the rectal gland cell via a urea transporter[4]. In the present series of experiments we re-examined the effect of lowering the concentration of urea in the perfusate of isolated rectal glands and also the effect of the inhibition of the urea transporter with phloretin, on the urea content of rectal gland cells and the secretion of chloride and urea.

Isolated rectal glands of *S. acanthias* were perfused through their single artery by gravity at 16˚C and 40 mm Hg pressure with oxygenated shark Ringer's solution containing 350 mM urea and 5 mM glucose in a single-pass perfusion. Glands were stimulated with theophylline 10^{-4} M and forskolin 5 x 10^{-8} M. In the experiments with phloretin, it was added at a final concentration of 10^{-4} M after the first thirty minutes of perfusion. Venous effluent and duct fluid were collected separately from PE-90 catheters placed in the vein and duct of the gland. tCollections were made every ten minutes. At the end of each experiment, an ~100 mg piece of the rectal gland was collected, cleaned of connective tissue and frozen in liquid nitrogen for tissue urea analysis. The tissues thus collected were homogenized in 10 volumes of 100 mM phosphate buffer pH 7.6. Chloride was measured using a Buchler-Cotlove chloridometer (Labconco, Kansas City, MO). Chloride secretion was calculated from the chloride concentration in the duct fluid, the volume of the fluid, the collection time, and the weight of the gland and expressed as μEq per gram of gland per hour. The urea concentration in the dogfish blood, perfusate, rectal gland tissue homogenates, and rectal gland secretion was measured using a commercially available urea kit (QuantiChrom™ Urea Assay Kit, Hayward, CA) and expressed as mmol/l. Plasma, perfusate and rectal gland secretion osmolality were measured using a water vapor osmometer (Wescor, Logan, Utah) and reported as mOsm/l. Protein concentration in tissue homogenates was measured using Bio-Rad Protein assay (Bio-Rad Laboratories, Hercules, CA). Statistical analysis was done using Student's *t*-test and ANOVA.

Reducing the concentration of urea in the perfusate resulted in a drop in the concentration of chloride in the secretion of the gland and also in significantly lower secretion of chloride, as shown in Figure 1. As expected, the osmolality of the secretion was also significantly reduced, as shown in Figure 2. The addition of phloretin to the perfusate also resulted in a reduction of the concentration of chloride in the secretion of the gland. This reduced concentration of chloride was associated with a drop in the amount of chloride secreted. However, there was no change in the osmolality of the rectal gland secretion as compared with that of control.

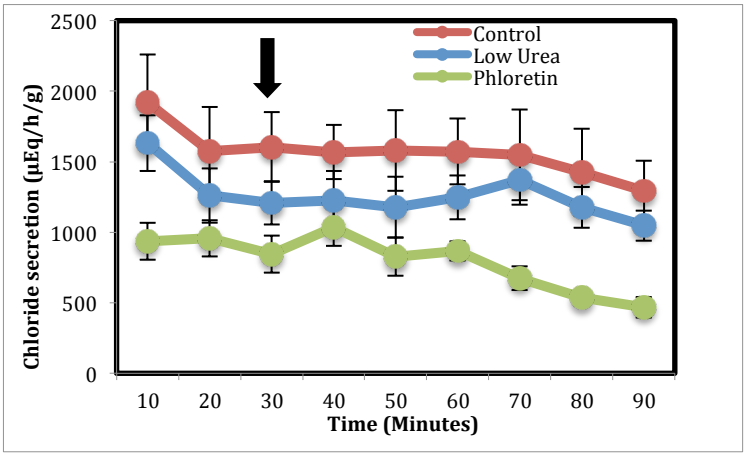

Figure 1. Effect of reducing urea in the perfusate and phloretin on the secretion of chloride by the rectal gland. Reducing the urea concentration in the perfusate to half 175 mM, caused a significant reduction in the secretion of chloride, $p < 0.001$ by ANOVA. Phloretin, 10^{-4} M added at the time indicated by the arrow also caused a significant reduction in the secretion of chloride, $p < 0.001$ by ANOVA. Data are expressed as mean ± SEM. N = 6 for all experiments.

The secretion of urea by these glands is shown in Figure 3. There was very little secretion of urea in the control gland perfused with 350 mM urea. Halving the urea concentration of the perfusate reduced the excretion of urea by the gland by approximately 27%, a statistical significant decrease by ANOVA, The addition of phloretin resulted in a progressive and significant increase in the amount of urea in the secretion of the gland as shown in the figure.

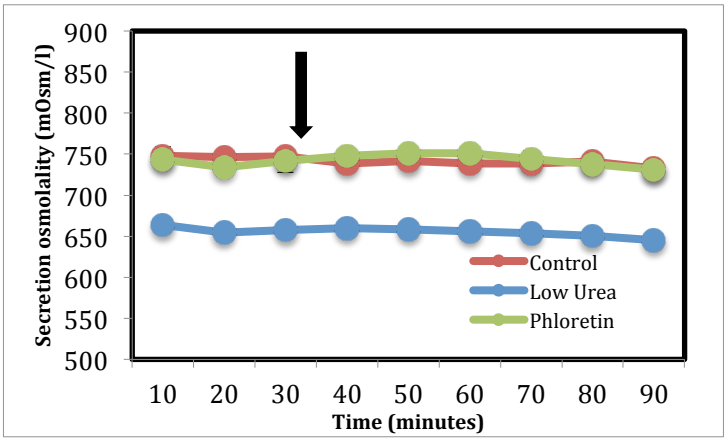

Figure 2. Effect of reducing urea in the perfusate on the osmolality of the rectal gland secretion. Halving the urea concentration the perfusate reduced significantly the osmolality of the secretion, $p < 0.001$ by ANOVA. Phloretin, 10^{-4} M added at the time indicated by the arrow, had no effect on the osmolality of the secretion. Points are mean ± SEM. Measures of dispersion cannot be seen because they are smaller than the symbols. N = 6 for all experiments.

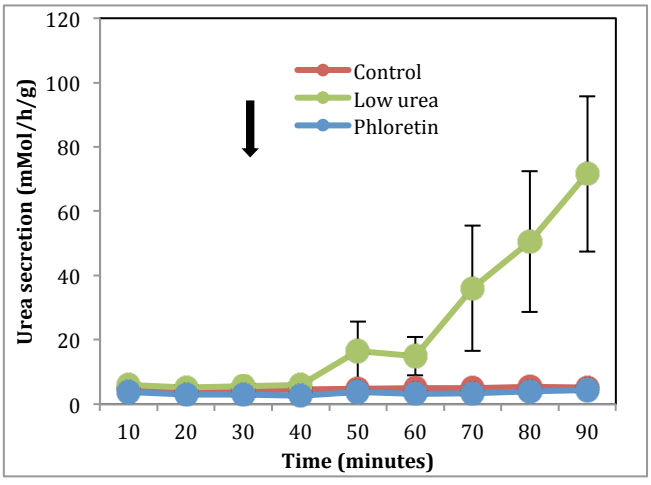

Figure 3. Effect of reducing the concentration of urea in the perfusate and phloretin on the secretion of urea by isolated perfused rectal glands. The secretion of urea that was low in the control experiments was further reduced by decreasing the concentration of urea in the perfusate to 175 mM, $p < 0.025$ by ANOVA. Phloretin 10^{-4} M added at the time indicated by the arrow, caused an unexpected rise in the urea secretion by the gland, $p < 0.0001$ by ANOVA. Points are mean ± SEM. Measures of dispersion cannot be seen in all the series because they are smaller than the symbols. N = 6 for all experiments.

The intracellular concentration of urea is shown in Figure 4. In the glands perfused with half the usual concentration of urea there was significant drop in its intracellular concentration to 32% of that seen in the glands perfused with 350 mM. Perfusions with phloretin, in the presence of 350 mM urea also resulted in a reduction of he intracellular concentration of urea to 62% of the control glands perfused with 350 mM urea.

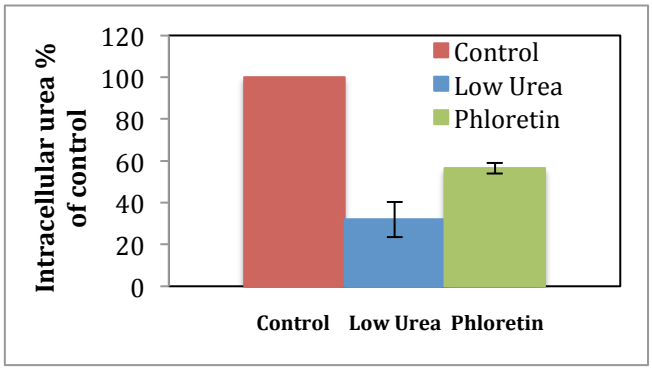

Figure 4. Effect of reducing the urea concentration in the perfusate of isolated perfused rectal glands and of phloretin on the intracellular urea concentration. Cutting in half the urea concentration of the perfsate reduced the intracellular urea concentration to 32% of control, $p < 0.001$ by Student's *t*-test. Phloretin, 10^{-4} M, significantly reduced the intracellular concentration of urea, $p< 0.001$ by Student's *t*-test. Columns are mean ± SEM. N = 6 for control and low urea and 4 for the phloretin experiments.

The observation that reducing the urea concentration in the perfusate of the isolated glands decreases the osmolality and the concentration of chloride of the secretion confirms pervious observations, and supports the notion that urea plays a role in the secretion of salt by the rectal gland. The fact that urea is present in only small amounts in the secretion of the gland that is isotonic with the plasma, allows the concentration of chloride to rise in the secretion and thus increase the total amount of salt excreted.

We have previously shown that rectal gland cells contain urea at the same concentration as that in the plasma or perfusate. Phloretin, an inhibitor of urea transporters, reduced the intracellular urea concentration of the rectal gland cells, as shown here. This observation suggests that the uptake of urea by the cells is mediated by a urea transporter present in the cell membrane. The functional effects of phloretin are also of interest. Sixty minutes after starting the perfusion of the gland with phloretin, the concentration of chloride in the perfusate dropped by 20%. In association with the drop in the concentration of chloride, the total secretion of chloride also dropped. These observations support the concept that urea, present in the plasma and the rectal gland cells, but not in the secretion of the rectal gland cells, allow the concentration of chloride in the secretion to be higher than that in the plasma. An interesting observation, yet unexplained, is that phloretin increases the concentration of urea in the secretion of the rectal gland. This unexplained phenomenon has been reported in another epithelia, the gills of the *S. acanthias*.[2]

1. **Burger JW, Hess WN.** Function of the Rectal Gland in the Spiny Dogfish. *Science*. 131: 670-671, 1960.
2. **Pärt P, Wright PA, Wood CM.** Urea and water permeability in dogfish (*Squalus acanthias*) gills. *Comparative Biochemistry and Physiology Part a, Molecular & Integrative Physiology*, 119, 117–123, 1998.
3. **Silva P, Stoff JS, Solomon RJ, Rosa R, Stevens A, Epstein J**. Oxygen cost of chloride transport in perfused rectal gland of *Squalus acanthias*. *J. Membr. Biol*. 53: 215-221, 1980.
4. **Silva P, Spokes KC, Kinne R.** The intracellular concentration of urea in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Isl. Biol. Lab*. 52:15-17, 2013.