

## The intracellular concentration of urea in the rectal gland of *Squalus acanthias*

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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 osmolality of the fluid secreted by the rectal gland is the same as that of the plasma. The composition is quite different. While the plasma of the shark contains sodium and chloride at a concentration of about 280 mmol/l and urea of 350 mmol/l, the fluid secreted by the rectal gland has a concentration of sodium and chloride that is almost twice that of the plasma with very little urea<sup>1</sup>. We and others have assumed the large difference in urea concentration between the plasma and the fluid secretion accounts for the almost doubling of the salt concentration in the gland secretion. In fact, such an assumption is supported by experiments that show that decreasing the concentration of urea in the perfusate of isolated glands decreases the concentration of salt in the secreted fluid<sup>2</sup>. Conversely, increasing the concentration of urea in the perfusate increases the concentration of salt in the fluid<sup>2</sup>. The low concentration of urea in the secretion of the gland indicates that the rectal gland cells represent a barrier for the diffusion of urea across the rectal gland epithelia. There is one published paper that reports that the basolateral membrane of the rectal gland cells is a selective barrier for urea<sup>3</sup>. There are no reports on the urea concentration in rectal gland cells. We examined this question by measuring the urea concentration in the rectal gland, and determining whether the rectal gland cell expresses a urea transporter.

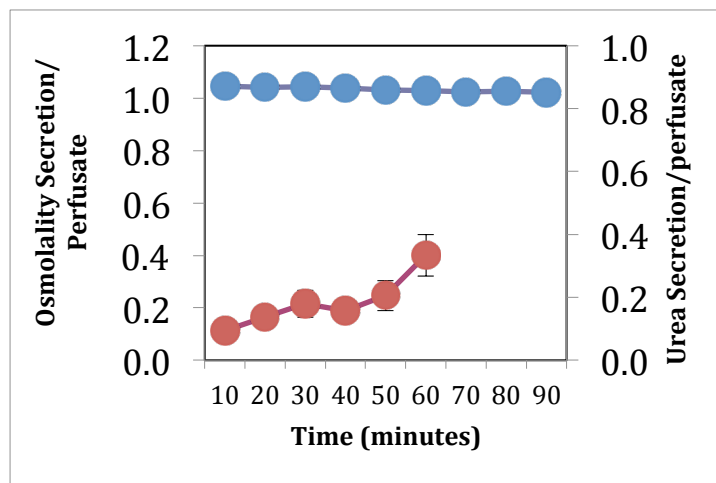
Isolated rectal glands of *S. acanthias* were perfused through their single artery by gravity at 16°C and 40 mm Hg pressure with oxygenated shark Ringer's solution containing 350 mM urea and 5 mM glucose in a single pass perfusion. Venous effluent and duct fluid were collected separately from PE-90 catheters placed in the vein and duct of the gland. Collections were made every ten minutes. Chloride was measured using a Buchler-Cotlove chlorhidrometer (Labconco, Kansas City, MO). Chloride secretion was calculated from the chloride concentration in the duct fluid, volume of the fluid, collection time, and weight of the gland and expressed as  $\mu\text{Eq}$  per gram of gland per hour. Urea concentration in dogfish blood, perfusate, rectal gland tissue, 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. To search for a urea transporter two rectal glands from a two dogfish were homogenized separately in lysis buffer from Qiagen using a Tekmar tissue homogenizer. The homogenates were passed through a Qiagen shredder column; messenger RNA was prepared using Qiagen RNAeasy minikit and treated with DNase. Single strand cDNA was then prepared with an Invitrogen First-Strand synthesis kit. PCR amplification was done using RedTaq ready mix from Sigma to search for urea transporter mRNA, using primers designed from known published sequences for elasmobranch urea transporter<sup>4</sup>. Statistical analysis was done using Student's *t* test.

Figure 1 shows the rectal gland fluid osmolality as ratio of the perfusate osmolality in perfused rectal glands over time. The ratio is about 1.0 and remains constant throughout the perfusion. Also shown in Figure 1 is the concentration of urea in the rectal gland secretion over time. The ratio secretion/perfusate urea is very low ranging from 0.1 to 0.4, indicating that the secretion of urea by the gland is very low.

Figure 2 shows the concentration of urea in mmol/l of tissue water in four different tissues of *S. acanthias*, rectal gland, kidney, muscle and brain. The concentration of urea is quite similar among the different tissues. The urea concentration for the different tissues are  $339 \pm 17$ ,  $n=8$ ,  $351 \pm 25$ ,  $n=8$ ,  $326 \pm 19$ ,  $n=17$ ,  $325 \pm 5$ ,  $n=14$ , for rectal gland, muscle, kidney, and brain, respectively, values are mean  $\pm$  SEM. All concentrations are corrected for tissue water content. The values for the rectal gland are corrected also for extracellular urea. The water

content and inulin space in the rectal gland has been previously measured and amount to 80% of the gland wet weight for the water content (unpublished data) and 27 to 30% for the inulin space<sup>5</sup>. The tissues water content for muscle, kidney, and brain was obtained from published reports<sup>6,7,8</sup>. The concentration of urea in the rectal gland secretion is more than an order of magnitude lower than that in the gland, therefore, the latter cannot contribute to the amount present in the gland cells. There were no significant differences among the different tissues. The urea concentrations in the tissues were similar to those in the plasma of the shark or perfusate of the rectal gland.

**Figure 1.** Ratios of rectal gland fluid secretion osmolality and urea concentration in the rectal gland. The ratio for osmolality was about 1.0 and remained constant during the length of the perfusion. The ratio for urea was approximately 0.1 at the beginning of the experiment rising to 0.4 by the end of the measurements. Symbols are mean  $\pm$  SEM; the SEM for the osmolality/perfusate ratio was smaller than the size of the symbol. The number of experiments is 24 for the first three timed collections and 12 for all subsequent collections.



**Figure 2.** Urea concentration in rectal gland, muscle, kidney and brain is shown here. The concentration of urea is quite similar among the different tissues and is not different from that in the perfusate of the glands. Values represent mean  $\pm$  SEM. Number of measurements was 8, 8, 17, and 14 for rectal gland muscle, kidney and brain, respectively. The values were not significantly different by “t” test, among the four different tissues

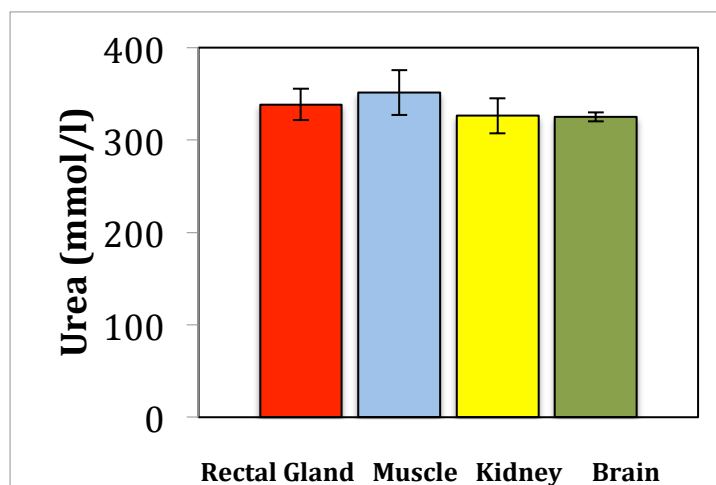


Figure 3 shows the sequence obtained using PCR for the urea transporter with the primers designed for it. The sequence is 99% homologous to that of other elasmobranch urea transporters found in kidney from *S. acanthias*, *S. canicula*, *T. scyllium*, *C. milli* and colon of *H. collei*.

**Figure 3.** Partial nucleotide sequence of a urea transporter in the rectal gland of *S. acanthias*. The sequence contains 322 bases.

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TCAGATGGCTGTGTTTCGTGTCAAAGGAGATTG
GTATTGGTGGCTGCTGCCGCCTGTGATCGTCAT
GTCAATGGCGTGCCCTGTGCTTTCAGTGCCTT
GGCCTGCTGTGATGGAAAAATTGGGGATCTGCC
TGTTTTTCACTCTGCCTTTTAATCATTCGTCGT
GTGCCGTGTACATGGCCTGCAACAGGACACGAC
AACCAGCATTTTCCCTCAGGTTCTTATTCAACC
CATGACTTCACCATTAAACACTACATGGCCTGA
GCTCAGTAGTTTCAATGCTTCTGAGAGCTATCC
CAGTGGGTGTTGGGCCTGGTGTATG
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The observation that the concentration of urea in the rectal gland is similar to that of the plasma or perfusate while that in the rectal gland secretion is much lower indicates that in the rectal gland epithelia the barrier for urea is at the apical membrane. A previous report has indicated that the barrier for urea in the rectal gland is located on the basolateral membrane<sup>3</sup>. This conclusion was arrived at by measuring the differential permeabilities for urea and water in plasma membrane vesicles prepared from rectal gland and separated by differential centrifugation into basolateral and apical vesicles. That report indicated that the permeability for urea in the basolateral membranes was lower than that expected given the permeability for water, but that does not mean that the basolateral membrane of the rectal gland has no permeability to urea. Thus, urea could enter the cell via the basolateral membrane more slowly than water, but given the large amount of basolateral membrane due to the numerous infoldings on the membrane, urea could equilibrate between the extracellular and intracellular space. Harder to explain is the finding the urea and water permeabilities are the same in the apical membrane that according to the results reported here must be the site for a barrier for urea.

The finding that the rectal gland expresses a urea transporter suggests that rectal gland has the necessary means to transport urea effectively across plasma membranes. It is tempting to assert that the urea transporter is located on the basolateral membrane, but at present we do not have evidence about either its location or abundance.

In summary, urea is equilibrated across the basolateral cell membrane of the rectal gland. The movement of urea into the rectal gland cell is mediated by a urea transporter that is only partially characterized. The movement of urea into the ducts of the rectal gland appears to be significantly limited suggesting that the apical membrane does not have a urea transporter. Urea reaching the rectal gland secretion must travel via another pathway.

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