INHIBITION OF GLUTAMINE SYNTHETASE INCREASES BRANCHIAL AMMONIA EXCRETION BY THE DOGFISH, *SQUALUS ACANTHIAS*

Gregg A Kormanik
Department of Biology, Univ. North Carolina at Asheville, NC 28804;

Urea synthesis by elasmobranchs uses glutamine synthesized from ammonia via glutamine synthetase (Anderson, P., Science 208:291-293, 1980). Ammonia excretion is low in *Squalus* embryos (Kormanik, G., J. Exp. Biol. 144:583-587, 1989) and adults, compared to that of seawater teleosts (Wood, C., P. Part and P. Wright, J. Exp. Biol. 198:1545-1558, 1995). We have shown that enzyme ratios favor glutamine synthesis in liver, brain, kidney and gill, and that the gill is the major site for ammonia excretion (Kormanik et al., Bull. MDIBL 37:99-100,.1998; Kormanik et al., Bull. MDIBL 39:102-103, 2000). Ammonia may be retained at excretory surfaces by conversion to the far less permeable glutamine molecule via glutamine synthetase. To determine if glutamine synthetase plays a role in ammonia retention, ammonia and urea excretion via gills and kidney were measured in *Squalus acanthias* before and after injection of L-methionine sulfoximide, an inhibitor of glutamine synthetase (Kormanik et al., Bull. MDIBL 39:102-103, 2000). Male dogfish (*Squalus acanthias*) were obtained and prepared as previously described (Kormanik et al., Bull. MDIBL 39:102-103, 2000). Fish (1-2 kg) were placed in covered, aerated 16.5 liter fiberglas boxes with running seawater (14 C.) and an air-lift recirculator, and allowed to recover for 24 hours. Flow to the box was stopped for a 2 hour control period, the box was rinsed (0.5 hrs) and L-methionine sulfoximide (72 mg/kg in 1-2 ml Elasmobranch Ringer's solution) was injected via an indwelling cannula. Flow was again stopped for 2 hour periods, with intermediate rinsing. Water samples representing branchial efflux were collected for ammonia and urea flux measurement and analyzed as previously described (Kormanik et al., Bull. MDIBL 39:102-103, 2000). Date were analyzed using a one-way ANOVA on ranks (Dunn's test). Data are expressed as mean ± SEM. Results of the experiments are shown in Table 1. Branchial excretion rates for ammonia increased significantly over time ($p=0.030$) while those for urea did not ($p=0.325$; urea rates not shown). Thus branchial glutamine synthetase may help prevent nitrogen loss at the gill by converting ammonia nitrogen to the less permeable nitrogen carrier glutamine, as well as provide nitrogen for urea synthesis.

Table 1. Ammonia (total: $NH_3 + NH_4^+$) excretion by the gills of *Squalus acanthias* before (control) and after injection of the GSase inhibitor, methionine sulfoximine (n =2- 8). Excretion is expressed in micromol kg^{-1} h^{-1}. Post-injection rates are significantly different from the control period ($p=0.030$).

		|----------Post Injection Period (hour)----------|						
	Preinjection Control	1	2	3.5	4.5	6	7	8.5	24
Ammonia	56.7 ± 14	45.0 ± 13	103 ± 29	112 ± 34	129 ± 61	224 ±137	229 ±64	277 ±148	213 ±77

Supported by NSF IBN 9507456 and a UNCA Intramural Grant to GAK.