INHIBITION OF GLUTAMINE SYNTHETASE AND ITS EFFECT ON AMMONIA EXCRETION BY THE DOGFISH, *SQUALUS ACANTHIAS*

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Ammonia provides nitrogen for urea synthesis by elasmobranchs, and 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). These observations suggest that permeability of excretory surfaces for ammonia is low, and ammonia may even be retained at excretory surfaces by conversion to the far less permeable glutamine. Urea synthesis by elasmobranchs uses glutamine synthesized from ammonia via glutamine synthetase (Anderson, P., Science 208:291-293, 1980) . The ratio of glutamine synthetase to glutaminase activity in various tissues can indicate the balance between glutamine synthesis and degradation, and thus indentify potential sites for net production of glutamine (Chamberlin, M. and J. Ballantyne, J. Exp. Zool. 264:267-272, 1992). We have shown that enzyme ratios favor glutamine synthesis in liver, brain, kidney and gill; the latter two are sites for ammonia excretion (Kormanik et al., Bull. MDIBL 37:99-100, 1998). To determine if glutamine synthetase (GSase) plays a role in ammonia retention, we measured the degree of inhibition of GSase *in vitro* by methionine sulfoximine in order to determine a sufficient dose for use in *vivo*. We then measured ammonia excretion via gills and kidney in *Squalus acanthias* before and after infusion of the inhibitor.

Male dogfish (*Squalus acanthias*) were obtained from Frenchman Bay by a commercial collector and maintained in floating live cars until used in experiments. Animals were killed by pithing, and tissues collected for the *in vitro* tissue enzyme assays as previously described (Kormanik et al., Bull. MDIBL 37:99-100, 1998). Methionine sulfoximide (L- and DL-, Sigma Chem. Co.) was added to yield 0.2 to 4 mM. For the *in vivo* experiments, fish (1-1.5 kg) were anesthetized with MS-222 (1:2000). A cannula (PE 60) was installed in the caudal blood vessel and the urinary papilla was catheterized to drain urine using PE 90 tubing, a purse-string ligature and cyanoacrylate glue to prevent slippage. Fish were placed in a covered, aerated 16.5 liter fiberglas box with running seawater (14 C.) 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 methionine sulfoximide was injected via the cannula. Flow was again stopped for 3 hours. Water and urine samples were collected hourly for ammonia and urea flux measurement. Water was analyzed for urea using Sigma Kit #535. Ammonia in seawater was analyzed using the method of Verdouw et al. (Water Research 12:399-402, 1978). ANOVA and Tukey's test were performed on the data using SigmaStat 2.0. Data are expressed as mean ± SEM.

The results of the *in vitro* enzyme inhibition experiments are shown in Table 1. Both L- and DL-isomer mix yielded inhibition data that were not significantly different, and thus were combined. Enzyme activity was inhibited to under 16% of the *in vitro* controls by 2.0 mM methionine sulfoximide, which was deemed a sufficient dose to use *in vivo* (i.e. 72 mg/kg in 1-2 ml Elasmobranch Ringer's, to yield 2 mM in extracellular fluid (ECF), assuming a 20% ECF volume).

This dose appeared to be well-tolerated by the fish, whose blood nitrogen parameters, appearance and behavior remained normal throughout the experiments. The results of the *in vivo* inhibition experiments are shown in Table 2. Urea nitrogen excretion predominates at both the gill and kidney. While gill ammonia excretion increases after injection in periods 2, 3 and 4, peaking in hour 3, it is not significant (ANOVA/Tukey Test, $p = 0.292$), presumably due to the variability of the data. Nor are there significant differences in kidney excretion after injection of the inhibitor.

Table 1. Enzyme activity *in vitro*, in the presence of DL-methionine sulfoximide (expressed as % of the uninhibited control [=100%], n = 5).

Inhibitor (mM)	0	0.2	0.4	1.0	2.0	4.0
Kidney	100	81.4 ± 5.1	45.5 ± 7.4	24.5 ± 6.4	13.6 ± 4.4	9.0 ± 2.6
Gill	100	75.5 ± 7.5	47.4 ± 15.5	32.3 ± 8.4	15.7 ± 7.4	6.3 ± 3.6

Table 2. Ammonia and urea excretion by the gills and kidney of *Squalus acanthias* before (control) and for four hourly periods after injection of the GSase inhibitor, methionine sulfoximine. Excretion is expressed in micromol kg^{-1} h^{-1}.

	Preinjection Control	Post Injection Period 1	2	3	4
Gill (n = 5)					
Ammonia	20.6 ± 6.9	17.5 ± 6.2	43.4 ± 20	51.0 ± 15	33.2 ± 8
Urea	504 ± 75	477 ± 53	574 ± 37	502 ± 22	565 ± 49
Kidney (n = 3)					
Ammonia	0.17 ± 0.06	0.84 ± 0.08	0.24 ± 0.16	0.22 ± 0.15	0.22 ± 0.17
Urea	17.5 ± 10.5	50.2 ± 38.0	16.9 ± 2.9	12.5 ± 6.1	24.2 ± 10.0

While 2 mM methionine sulfoximine is sufficient to inhibit GSase *in vitro*, it did not significantly affect ammonia excretion via either the gill or kidney *in vivo*. The intracellular enzyme may not have not been accessible to the inhibitor. The membrane permeability and the lifetime of methionine sulfoximine *in vivo* are not known. A higher concentration of inhibitor may be necessary, or more time for it to gain intracellular access to the enzyme. Experimental variability (at least for the gill excretion) obscured changes. Further experiments are required to better define the role of GSase in ammonia excretion and urea metabolism by the gills and kidney of *Squalus acanthias*.

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