Effects of thermal stress on xenobiotic transport by *Squalus acanthias* choroid plexus

Ashley N. Gordon[1], J. Larry Renfro[2], David S. Miller[3], Alice R.A. Villalobos[4]

[1]Biological Studies Dept., Univ. of Maryland, Baltimore County, Baltimore, MD 21250
[2]Dept. of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269
[3]Lab. of Pharmacol. and Chemistry, NIEHS/NIH, Research Triangle Park, NC 27709
[4]Dept. of Environmental Medicine, University of Rochester, Rochester, NY 14642

Our objective is to determine the effects of thermal stress, in the presence and absence of the endogenous cytoprotectant, trimethylamine oxide (TMAO), on organic anion transport across the blood-cerebrospinal fluid (CSF) barrier of the spiny dogfish shark. Prior work has indicated that increased levels of heat shock proteins (HSPs) may alter transepithelial transport[1]. TMAO stabilizes protein tertiary structure, and we hypothesize that it may alter the heat shock response.

Active transport of ^{14}C-2,4-dichlorophenoxyacetic acid (2,4-D), an organic anion and commonly used pesticide, was measured in both freshly isolated and cultured shark IVth plexuses subjected *in vitro* to mild heat stress (MHS, 18.5°C for 6 h) or severe heat shock (SHS, 23.5°C for 1 h). The cultured tissues were incubated at 13.5°C for at least 48 h in sterile L-15 culture medium modified with urea and TMAO for use with elasmobranch tissues[3]. Other tissues were identically treated but in the absence of TMAO, which was replaced with an equivalent concentration of urea. Freshly isolated tissues were placed in shark Ringer and immediately stressed upon removal from the animal. All stressed tissues were given a recovery time of 1.5 h at 13.5°C prior to determination of fluxes and electrical properties (transepithelial potential difference and electrical resistance) in Ussing chambers. The recovery time allowed for accumulation of HSPs.

Table 1. Effects of heat stress on unidirectional blood-side to CSF-side (B to C), CSF-side to blood-side (C to B), and net fluxes of ^{14}C-2,4-D (10 μM) across the short-circuited shark choroid plexus.

Treatment	B to C	C to B	Net	B to C	C to B	Net
Cultured	*No TMAO*			*72 mM TMAO*		
Control	0.34 ± 0.063	-0.70 ± 0.145	-0.37 ± 0.159	0.47 ± 0.259	-0.78 ± 0.296	-0.31 ± 0.112
MHS	0.38 ± 0.221	-0.61 ± 0.283	-0.23 ± 0.145	0.30 ± 0.068	-0.55 ± 0.023	-0.26 ± 0.073
SHS	0.14 ± 0.078	-0.49 ± 0.098	-0.35 ± 0.095	0.86 ± 0.461	-0.94 ± 0.384	-0.08 ± 0.112[a]
Freshly Isolated						
Control	----	----	----*	0.16 ± 0.068	-0.45 ± 0.109	-0.29 ± 0.061
MHS	0.00 ± 0.074	-0.29 ± 0.159	-0.28 ± 0.156	0.00 ± 0.094	-0.35 ± 0.012	-0.44 ± 0.083
SHS	0.33 ± 0.090	-1.02 ± 0.169	-0.69 ± 0.196[b]	0.32 ± 0.103	-0.94 ± 0.185	-0.62 ± 0.226[b]

Values are means ± SEM (n = 4-9). Fluxes are *nmoles x cm^{-2} x h^{-1}* determined after 1 h in Ussing chambers. *Freshly isolated controls were not tested in absence of TMAO. [a] Significantly different from controls cultured with or without TMAO. [b] Significantly different from freshly isolated controls in presence of TMAO.

Table 1 shows the effects of MHS (Δ5°C for 6 h) and SHS (Δ10°C for 1 h) on unidirectional secretory (blood-side to CSF-side, B to C), reabsorptive (C to B) and net fluxes of ^{14}C-2,4-D. Active net reabsorption was maintained at freshly isolated tissue control (+TMAO) levels following 48 h in culture with or without TMAO. MHS had no effect whether tissues were maintained with or without TMAO as compared to controls. In freshly isolated tissues, SHS in the absence of TMAO slightly stimulated reabsorption but had no effect in tissues subjected to SHS in the presence of TMAO. Most notably, net active reabsorption was reduced to near zero in SHS tissues cultured with TMAO but was

unaffected by SHS in tissues cultured without TMAO. Heat stress had no effect on electrical properties in any of the tissues. The cytoprotectant, TMAO, may suppress the denaturation of protein, decreasing the major signal (slightly denatured protein)[2] for increased HSP production during the 48 h culturing period. Thus, potentially more damage may occur in TMAO 'protected' tissues during subsequent SHS.

Supported by NSF IBN-0078093, NIH-NIEHS-ES10469, NIH-NINDS-NS39452, NIH-NIEHS-P30-ES01247 and CMTS (NIEHS P30-ES3828). Ms. Gordon was an REU students (NSF DBI-0139190).

1. Brown, M. A., R. P. Upender, L. E. Hightower, and J. L. Renfro. Thermoprotection of a functional epithelium: Heat stress effects on transepithelial transport by flounder renal tubule in primary monolayer culture. *Proc. Natl. Acad. Sci. USA* 89: 3246-3250, 1992.
2. Hightower, L. E. Heat shock, stress proteins, chaperoning and proteotoxicity. *Cell* 66: 191-197, 1991.
3. Villalobos, A. R. A., D. S. Miller, and J. L. Renfro. Transepithelial organic anion transport by shark choroid plexus. *Am J Physiol Regul Integr Comp Physiol* 282: R1308-1316, 2002.