ION DEPENDENCE OF ORGANIC ANION TRANSPORT ACROSS DOGFISH SHARK (*Squalus acanthius*) CHOROID PLEXUS

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The choroid plexus forms the blood-cerebrospinal fluid (CSF) barrier and in many respects is the "kidney of the brain." Together with the brain capillaries, i.e., the blood-brain barrier, choroid plexus regulates the environment of the CNS by absorbing nutrients from the blood and removing xenobiotics, xenobiotic metabolites and metabolic wastes from the brain for subsequent excretion in bile and urine. Like renal proximal tubule and liver, choroid plexus possesses a potent excretory transport system for organic anions, including neurotransmitter metabolites (5-hydroxyindoleacetic acid), drugs (penicillin), and environmental toxicants (2,4-dichlorophenoxyacetic acid, 2,4-D).

Transepithelial absorption of organic anions from CSF to blood is a three-step process: ventricular (apical) uptake from CSF, transcytosis, and basolateral efflux into the subepithelial/vascular space. Models of organic anion transport by mammalian choroid plexus and renal proximal tubule predict indirect Na^+-gradient dependence of apical uptake and electrical potential dependence of basolateral efflux (Breen et al. Am. J. Physiol. 282: F877-F885, 2002). Because of its favorable neuroanatomy, dogfish shark IVth plexus can be mounted in flux chambers for measurement of unidirectional tracer fluxes (using 10 μM ^{14}C-2,4-D) under biophysically defined conditions, i.e., absence of chemical and electrical gradients. In addition, transport of fluorescent organic anions (1 μM fluorescein, FL) in tissue from the lateral and IVth plexus can be imaged by confocal microscopy. Our initial flux chamber and imaging studies have shown that organic anion (2,4-D) absorption from CSF to blood is active, specific and ouabain-sensitive (Villalobos et al. Am. J. Physiol. 282: R1308-R1316, 2002). As in mammalian choroid plexus (Breen et al. *op. cit.*; Sweet et al. J. Biol. Chem. 277: 26934-26943, 2002), transepithelial transport in shark choroid plexus involves concentrative steps at both the apical and basolateral membranes (Villalobos et al. *op. cit.*).

In control experiments with IVth plexus mounted in flux chambers, the average ratio of absorptive (apical to basal) to secretory (basal to apical) 2,4-D flux was about 6 (for detailed methods, see, Villalobos et al, *op. cit.*). The organic anion, probenecid (0.1-1 mM), nearly abolished net absorption of 2,4-D. When control Ringer's solution (280 mM NaCl) was replaced with low-Na Ringer's (10 mM NaCl, remainder replaced with N-methyl-D-glucamine) at the ventricular side, absorptive flux fell by 50% and secretory flux doubled; as a result, net flux fell by 90%. Similarly, in confocal microscopy studies, reduction of external Na inhibited FL transport. In control tissues, FL accumulated in the epithelial cells and the subepithelial and vascular spaces with the fluorescence gradient being subepithelial/vascular space > epithelial cell

> medium. However, when tissues were incubated in low Na Ringer's solution, fluorescence intensities in the cells and subepithelial and vascular spaces decreased by 70-80%.

Organic anion uptake in mammalian renal proximal tubule and choroid plexus is energetically indirectly coupled to the flux of Na. Cellular uptake of organic anion via organic anion/α-ketoglutarate exchange is coupled to lithium-sensitive Na-driven accumulation of the Kreb's cycle intermediate, α-ketoglutarate (Pritchard and Miller Physiol. Rev. 73: 765-796, 1993; Pritchard et al. J. Biol. Chem. 274: 33382-33387, 1999.). Thus, Li-sensitivity of organic anion transport is indicative of indirect coupling to Na transport. In shark IVth choroid plexus mounted in flux chambers, 5 mM LiCl tended to decrease 2,4-D absorptive flux and increase secretory flux, the result being a 40% decrease in net 2,4-D flux. In confocal imaging experiments, 10 mM LiCl reduced FL accumulation in cells and subepithelial/vascular spaces by 70 % and 90%, respectively.

In rat choroid plexus, elevation of extracellular K increases cellular accumulation of FL and blocks efflux of substrate into the subepithelial/vascular spaces (Breen et al. *op. cit.*). This was taken as evidence that basolateral efflux was driven by electrical potential difference (PD). In shark, 2,4-D fluxes were also sensitive to elevated medium K. Increasing extracellular K from 3 mM to 30 mM decreased both absorptive and secretory fluxes; the net flux fell by 90%. FL transport was similarly affected by increased extracellular K; cellular uptake increased nearly 4-fold, and subepithelial/vascular accumulation decreased by 70%.

Together, the present flux and imaging data indicate that organic anion transport across shark choroid plexus is dependent on the ionic composition of the bathing medium. Reducing medium Na reduced transepithelial transport by inhibiting, at a minimum, uptake of substrate at the apical membrane. Increasing medium K reduced transepithelial transport apparently by blocking efflux at the basolateral membrane. Thus, as in mammalian tissue, organic anion transport across shark choroid plexus involves Na-dependent uptake followed by PD-driven efflux. Uptake may be mediated by a shark orthologue of Oat3, which in mammals mediates organic anion uptake via indirect coupling to the Na gradient (Nagata et al. Mol. Pharmacol. 61: 982-988, 2002; Sweet et al. Am. J. Physiol. in press, 2003; Miller et al. unpublished data). Studies are underway to identify and characterize at the molecular level the organic anion transporter(s) in shark choroid plexus. Supported by NSF IBN-0078093, NIH-NIEHS-ES10469, NIH-NINDS-NS39452, NIH-NIEHS-P30-ES01247 and the Center for Membrane Toxicity Studies (NIEHS P30-ES3828). Ms. Unites and Ms. Gordon were REU students (NSF DBI-0139190).