Regulation of organic anion transport in the choroid plexus of spiny dogfish shark, *Squalus acanthias*

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The choroid plexus epithelium forms the blood-cerebrospinal fluid (CSF) barrier, which along with the blood-brain barrier, maintains the fluid environment of the brain. The choroid plexus not only secretes CSF but also transports potentially toxic xenobiotics and waste products of neural metabolism to the blood for eventual clearance in the kidney and liver. In this regard, previous studies have shown that choroid plexus, like kidney, actively transports organic anions[4]. For the large, fluorescent organic anion, fluorescein-methotrexate (FL-MTX), similar systems appear to drive CSF to blood transport in tissue from mammals and dogfish shark. Two carrier-mediated steps in series are involved, with uptake at the apical plasma membrane (CSF-side) being Na-dependent and efflux at the basolateral membrane (blood-side) being highly concentrative[1,2]. Although we are beginning to obtain a molecular level understanding of organic anion transport in choroid plexus[4,5], little is known about how such transport is regulated. The present study was initiated to identify cellular signals that modulate FL-MTX transport in shark choroid plexus.

Transepithelial transport of FL-MTX across isolated lateral choroid plexus from dogfish shark was studied using confocal laser scanning microscopy (Olympus) and quantitative image analysis (Scion Image software) as described previously[1-3]. This procedure allowed us to measure the steady state (60 min) distribution of FL-MTX within the tissue and quantify levels in the epithelial cell and subepithelial/blood vessel compartments. In control tissue incubated in medium with 2 μM FL-MTX, cellular fluorescence was somewhat lower than medium fluorescence, but subepithelial/blood vessel was about 5 times higher than both. FL-MTX accumulation in both compartments was abolished when probenecid, a potent inhibitor of organic anion transport, was added to the medium.

In renal proximal tubule, organic anion transport is stimulated by a mitogen-activated protein kinase pathway and inhibited through activation of protein kinase C (PKC)[6]. In shark choroid plexus, phorbol ester, which activates PKC, significantly reduced FL-MTX accumulation in both cellular and subepithelial/blood vessel compartments; these effects were blocked by bisindolylmaleimide, a PKC-selective inhibitor. In addition, forskolin, which activates PKA increased FL-MTX accumulation in both cellular and subepithelial/blood vessel compartments by more than 50%; this effect was blocked by H89, a specific inhibitor of PKA. Time course studies with forskolin showed rapid activation of apical FL-MTX uptake followed by increased subepithelial/blood vessel accumulation. Thus, in shark choroid plexus, protein kinase-based mechanisms are in place to both increase and decrease transport, presumably in response to hormonal exposure. The hormones that actually act through these protein kinases to modulate organic anion transport remain to be identified. This study was supported by grant GF 1211/12-1 of the German Research Foundation (GF), the MDIBL Center for Membrane Toxicity Studies and the Howard Hughes Medical Institute (KDD).

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