Insulin regulation of organic anion transport across the choroid plexus of dogfish shark, *Squalus acanthias*

Heike Gutmann[1], Juergen Drewe[1], David S. Miller[2]

[1] Institute of Clinical Pharmacology and Toxicology, Dept. of Research, University Clinic Basel, Switzerland

[2] Laboratory of Pharmacology and Chemistry, NIH/NIEHS, Research Triangle Park, NC, 27709

The choroid plexus removes potentially toxic xenobiotics and metabolic wastes from the cerebrospinal fluid (CSF) into the blood. The molecular basis for transport is the polarized expression of several multispecific transport proteins on the apical (CSF-side) and basolateral (blood-side) membranes of the choroid plexus epithelium. In mammals, members of the organic anion transporter (Oat), organic anion transporting polypeptide (Oatp), and multidrug resistance-associated protein (MRP) families have been implicated[1,2]. In dogfish shark choroid plexus, transport of the large, fluorescent organic anion, fluorescein-methotrexate (FL-MTX) from CSF to blood involves two steps: Na-dependent uptake at the apical membrane and potential-driven efflux at the basolateral membrane; Oat-like, Oatp-like and Mrp-like transporters have been implicated[3]. These experiments also showed that transepithelial FL-MTX transport decreases when protein kinase C (PKC) is activated and increases when PKA is activated. In the present experiments, we begin to investigate the hormonal basis for this signaling as well as other intracellular signals that affect transport.

Accumulation of FL-MTX in the cells and blood vessels of lateral choroid plexus from dogfish shark was measured using confocal laser scanning microscopy and quantitative image analysis (ImageJ software) as described previously[3]. In initial experiments, we found that transport was not affected by micromolar concentrations of the polypeptide hormones glucagon, IGF-1 and EGF. In contrast 0.25-1 μM insulin significantly reduced transepithelial FL-MTX transport. Bisindolylmaleimide (BIM, 100 nM), a potent, PKC-selective inhibitor, blocked the insulin-induced reduction of FL-MTX transport; as previously shown[3], BIM alone did not affect FL-MTX transport. Transepithelial transport of FL-MTX was also reduced by vanadate, a phosphatase inhibitor. The effect of vanadate was partly blocked by BIM. Wortmanin, an inhibitor of PIP3-kinase, PD98059, an inhibitor of MAPK2/ERK2, as well as L-NMMA, an inhibitor of NO synthase, did not block the vanadate-induced reduction of FL-MTX transport. This is the first report of hormonal regulation of organic anion transport in choroid plexus from any species. The data indicate that insulin signaling through PKC is independent of the mainstream MAP kinase pathway. In addition, the high concentrations of insulin needed to reduce transport suggest action is not through a insulin receptor.

1. **Breen, C., Sykes, D.B., Fricker, G. and D.S. Miller**. Confocal imaging of organic anion transport in intact rat choroid plexus. *Am. J. Physiol.*, 282:F877-F885, 2002.
2. **Breen, C.M., Sykes, D.B., Baehr, C., Fricker, G., and D.S. Miller**. Fluorescein-methotrexate transport in rat choroid plexus analyzed using confocal microscopy. *Am. J. Physiol.*, 287:F562-F569, 2004.
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