REGULATION OF XENOBIOTIC EFFLUX PUMPS IN KILLIFISH (*FUNDULUS HETEROCLITUS*) BRAIN CAPILLARIES

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The brain capillary endothelium is a formidable barrier to the entry of xenobiotics into the central nervous system (CNS). This barrier protects the CNS from toxic chemicals, but also denies entry to therapeutics, e.g., chemotherapeutics. Miller et al (Mol. Pharm. 58:1357-1367, 2000) developed a simple but powerful experimental system in which to study drug transport across intact brain capillaries. It consists of freshly isolated capillaries, fluorescent substrates and confocal imaging. This system was used to follow bath to lumen transport of selected fluorescent xenobiotics in capillaries from rat and pig where transport was found to be mediated by two ATP-driven drug export pumps, p-glycoprotein and Mrp2. One shortcoming of the system was the limited capillary viability. We recently validated a new and long-lived comparative model for studying drug transport across the blood-brain barrier (Miller et al, Am. J. Physiol., 282:R191-R198, 2002). Using brain capillaries isolated from two poikilotherms, a teleost (killifish) and an elasmobranch (dogfish shark, *Squalus acanthias*) we found, as in mammals, evidence for involvement of two ATP-driven xenobiotic export pumps, p-glycoprotein and Mrp2, in transport from CNS to blood. As is mammals, both transporters were localized to the luminal membrane of the endothelial cells. The present report documents initial attempts to identify mechanisms that alter transport function in killifish brain capillaries.

Accumulation (60 min) of fluorescent substrates in individual killifish capillary lumens was measured using confocal microscopy and quantitative image analysis. Substrates used were sulforhodamine 101 free acid for Mrp2 and a fluorescent verapamil derivative for p-glycoprotein. Cell to lumen transport by both drug efflux pumps was reduced by treatments that elevated intracellular cyclicAMP, cyclicGMP and nitric oxide (NO) and that activated protein kinase C (PKC). A PKC-selective inhibitor, blocked the effects of PKC activation. The polypeptide hormone, endothelin-1 (ET-1), at low nanomolar concentrations, also reduced transport. ET-1 appeared to act through an ET_B receptor and at least in part through NO synthase and NO. ET-1 did not act through PKC, PKA or PKG, suggesting that additional hormones could acutely alter transport function by signaling through parallel pathways. None of the treatments that reduced luminal accumulation of the fluorescent substrates increased passive permeability to small fluorescent dextrans, suggesting that reduced xenobiotic efflux pump activity rather than increased tight junctional permeability underlies the effects observed.

The present initial results indicate that at least four parallel signaling pathways (ET-NO, PKC, PKA, PKG) regulate Mrp2 and p-glycoprotein function in brain capillary endothelial cells. Since these xenobiotic export pumps are believed to be important constituents of the "active" blood-brain barrier, understanding how signaling is turned on and off could be of value in designing treatments to strengthen a damaged barrier and to overcome the barrier and deliver therapeutics to the CNS. Supported in part by a travel grant from the Maren Foundation to BB, by DFG FR1211/8-1 to GF and by the MDIBL CMTS (ES 03828).