Blood-brain barrier transposition by VIP $VPAC_1$ selective ligands

Place of work: Epilepsy and Aging Lab, GER- Gene expression and Regulation and FunGP-Functional genomics and proteomics at BioISI.

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Abstract / MSc thesis project proposal:

Vasoactive intestinal peptide (VIP) $VPAC_1$ receptors (Rs) regulate synaptic plasticity in both physiological and pathological conditions, such as pro-epileptogenic events[1–3]. This has implications to brain neuroprotection. Exogenous VIP was reported to cross the rat blood-brain barrier (BBB) *in vivo* through a non-saturable mechanism (likely transmembrane diffusion)[4]. Other peptides in the VIP-PACAP-secretin family cross the BBB by peptide transporter 6 (PepT6)[5]. Selective $VPAC_1$ R ligands (agonists and antagonists) are chimeric peptides based on the sequence of peptides in this family and may well benefit from the same ability to cross the BBB[2]. This project will use cultures of rat brain microvascular endothelial cells (RBMVECs) and BBB spheroids (3D culture) to investigate the ability of $VPAC_1$ R ligands to cross the BBB and further investigate the mechanisms of VIP BBB transposition.

RBMVECs will be obtained from the rat brain as described[6] and seeded on rat-tail collagen-I coated polyester transwell inserts (0.4 µm; 8×10^4 cells/insert) for BBB transposition studies and in 96-well plates, with no inserts, over an extracellular matrix coat when studying peptide cell accumulation. Establishment of the BBB will be assessed by transendothelial electrical resistance (TEER) measurements (~10-15 days). At this stage, the ability of VIP (positive control) or two $VPAC_1$ selective ligands (PG 97-269, a $VPAC_1$ R antagonist, or [K^{15}, R^{16}, L^{27}] VIP (1-7)/GRF (8-27), a $VPAC_1$ R agonist) to cross the cell layer or to enter the cells will be evaluated by quantifying the presence of peptides in the lower culture media compartment by mass spectrometry (MS) or with a fluorescent plate reader (upon sample concentration) by using FAM/FITC-labelled peptides. These will also be used to monitor accumulation of $VPAC_1$ ligands within cells using confocal microscopy. The presence of VIP $VPAC_1$ receptors PepT6, vascular cell adhesion molecule 1 (VCAM), zonula occludens-1 (ZO-1) or occludin-5 and -1 may also be evaluated by immunocytochemistry. A BBB spheroid 3D culture will be used to confirm and refine the results obtained.

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