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

Place of work/: Epilepsy and Aging Team, BioISI – GER, FCUL & FunGP-Functional genomics and proteomics at BioISI.

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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) to investigate the ability of $VPAC_1$ R ligands to cross the BBB. BBB spheroids (3D culture) may be used to further investigate the mechanisms of VIP BBB transposition.

RBMVECs will be obtained from the rat brain as described(*6*) and seeded on transwell inserts (0.4 µm; 8×10^4 cells/insert) for BBB transposition studies and in 96-well plates over an extracellular matrix coat to study peptide cell accumulation. Establishment of the BBB will be assessed by trans endothelial 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 BBB or to enter the endothelial 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 inside the cells using confocal microscopy. The presence of VIP $VPAC_1$ receptors, PepT6 transporter, 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 may be used to confirm and refine the results obtained.

References:

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Alternative topics: (please contact the supervisor)**:**

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?
- Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM.
- Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels