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The role of PP2A in brain microvascular permeabiy

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Introduction: Tight junctions are regulated through the opposing actions of kinases and protein phosphatases [1, 2], the dysregulation of which is observed in multiple diseases. However, the role of protein phosphatase 2A (PP2A) in modulating permeability of the blood-brain barrier remains unclear. The present study investigates if PP2A alters brain microvascular permeability and expression of key junctional proteins.

Methods: Human brain microvascular endothelial cells (HBMECs) were exposed to okadaic acid (OA, 10nM), FTY-720 (5μM), histamine (10μM) or DMSO (0.01% v/v) for 24h. Transendothelial permeability was determined using FITC-dextran, while mRNA and protein expression were determined using PCR and immunoblotting. PP2Ac activity was determined using an immunoprecipitation assay. Data are presented as mean±S.E.M. (n=5) and were analysed by one-way ANOVA with *post hoc* (Bonferroni). * $P<0.05$.

Results: OA (PP2A inhibitor) elicited a fold increase in transendothelial permeability similar to that of histamine (positive control), while FTY-720 (PP2A activator) had no effect. OA increased ve-cadherin mRNA expression (~3 fold) but did not affect occludin or PE-CAM expression. OA reduced abundance of ve-cadherin and PE-CAM. Although mRNA expression was unaffected by FTY-720, abundance of ve-cadherin (2-fold) increased. OA decreased PP2A activity (58.9±5.46%) and PP2Ac mRNA expression (72.4±7.9%) without affecting protein expression. FTY-720 increased PP2A activity (50±6.82%) and PP2Ac mRNA expression (84.8±23.1%) without affecting protein abundance. OA increased abundance of demethylated PP2Ac, while reducing abundance of LCMT-1 (leucine carboxyl methyltransferase-1); abundance of PME-1 (protein phosphatase methylesterase 1) was not altered. FTY-720 did not alter methylated PP2Ac, LCMT-1 or PME-1 abundance. DMSO had no effect.

Conclusion: Pharmacological inhibition of PP2A increases permeability of the HBMEC monolayer consistent with loosening of the tight junctions due to decrease abundance of ve-cadherin and PE-CAM. This is associated with decreased PP2A catalytic activity, due to reduced methylation of the PP2A catalytic subunit, which affects assembly of the holoenzyme. The reduction in methylation is due to effects on LCMT-1 rather than PME1 abundance, which methylate and de-methylate PP2Ac respectively. This study implicates PP2A in regulating permeability of the blood brain barrier, and as such might be a viable target of therapeutic value.

References:

1. Bertocchi C *et al*. (2012). J Sig Transduct **2012**: 125295.2. Rao R (2009). Ann N Y Acad Sci **1165**: 62-68.