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Caveolae membrane system and astrocyte interactions within porcine brain microvascular capillary endothelial cell cultures

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Caveolae are classically defined as omega shaped invaginations of the plasma membrane enriched in cholesterol, sphingolipids, and their unique and functional protein, caveolin-1. Caveolae are abundant in many cell types including peripheral capillary endothelial cells, smooth muscle cells and type I pneumocytes, and are involved in the transport of solutes including macromolecules (Gumbleton et al 2000). Previous morphometric studies (Stewart 2000) determining the numerical density of caveolae-like vesicles within the Blood Brain Barrier (BBB) report a limited presence. Unlike clathrin coated pits, caveolae can exist in morphologically undetectable forms and a paucity of vesicles may not necessarily reflect functionality. Our aim is to investigate the function and modulation of the caveolae membrane system within the blood brain barrier. In our studies we utilise a primary culture of porcine brain microvascular endothelial capillary (PBMVEC) cells, isolated and cultured as previously described by our laboratory (Omidi et al 2003).

We report that while whole brain tissue (containing by volume 0.1% capillary endothelial cells) shows negligible levels of caveolin-1 expression, freshly isolated PBMVEC cells display high caveolin-1 expression comparable to model cell lines widely used in caveolae research i.e. MA104 & A431. Expression is not diminished in PBMVEC primary or 1st passage culture. In freshly isolated cells the level of phosphorylated-caveolin-1 is negligible while in primary and 1st passage cells the phosphorylation state is abundant. In non-brain endothelial cell types this change in phosporylation state is implicated in the endocytic potential of caveolae (Aoki et al 1999) and warrants further investigation.

Exposing PBMVEC cells to astrocyte (C6 glioma) conditioned media or astrocyte co-culture systems did not alter either the expression of caveolin-1 or its phosphorylation state in primary or 1st passage cells. Microscopy did not reveal any significant effect of C6 treatments upon vesicle density within the PBMVEC cells.

Whilst astrocyte derived factors do not appear to alter expression of caveolin-1 or its phosphorylation state, this does not mean that astrocyte derived factors do not modulate the caveolae biology of the BBB. The effect of astrocyte derived factors on caveolae function and solute transport is currently under investigation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Effect of supplementation with docosahexaenoic acid on P-glycoprotein function in an in vitro model of the brain endothelium

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The blood-brain barrier (BBB) is formed by cerebrovascular endothelial cells, under the inductive influence of astrocytic glia (Abbott, 2002). It maintains a homeostatic brain environment, by regulating molecular traffic between blood and brain. Closely apposed astrocytes provide fatty acids (FA) to the endothelium (Bernoud et al. 1998); this may modulate cell membranes and the expression of BBB features including tight junctions and transport proteins.

P-glycoprotein (Pgp) is a lipid-associated efflux transporter present in the BBB, which can prevent accumulation of toxic lipophilic agents (Romsicki and Sharom, 1999). We hypothesised that supplementation with the FA docosahexaenoic acid (DHA) would increase BBB Pgp activity in the rat brain endothelial cell line (RBE4); previous studies from our lab were contradictory, showing that 18μM and 73μM DHA in medium with 10% fetal calf serum (FCS) inhibited RBE4 Pgp function (transport assay), but increased Pgp expression (Western blot).

Control cells were cultured in 96 well plates in standard RBE4 medium with 10% FCS (Begley et al. 1996). For DHA supplementation (over 3 days), the serum level was reduced to 2% FCS, to lower the basal level of DHA, as FCS contains DHA. The effect of DHA on cellular Pgp activity was assessed by measuring uptake (volume of distribution, Vd) of the Pgp substrate [3H]-colchicine over 30 min (Begley et al. 1996).

DHA supplementation in the range 0.25-8μM caused a biphasic effect on Pgp activity. All the results were significant when compared to the 2%FCS control (ANOVA plus Tukey-Kramer post-test, $P<0.001$), with the Pgp activity maximal at 2μM DHA (Vd reduced from 39.03±0.08 to 6.55±0.26 μl/mg protein, mean ± S.E.M., n=6 wells for DHA, 12 for controls). Over a greater DHA range (1-64μM), only 1μM DHA increased Pgp function significantly ($P<0.05$).

In contrast to previous experiments, our DHA source was stored under nitrogen to reduce peroxidation. In an attempt to explain previous results, we stored the DHA under oxidising conditions (without nitrogen). Repeating the previous study (18μM and 73μM DHA) with DHA stored under nitrogen showed no significant increase in Vd. However DHA stored under oxidising conditions did significantly increase Vd at both 18μM and 73μM DHA ($P<0.0001$), suggesting that previous findings were due to the generation of toxic metabolites by DHA peroxidation.

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This study has implications for FA modulation of BBB transport, and FA supplementation as a way of optimising BBB function.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Evidence for bulk flow of brain interstitial fluid from 3-D image analysis of tracer-injected rat brain.

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Brain interstitial fluid (ISF) forms the microenvironment for neurones and glia. There is accumulating evidence that like cerebrospinal fluid (CSF), ISF is flowing not static (Abbott, 2004). The dynamics of ISF flow have important implications for CNS function. This study aimed to obtain new quantitative and anatomical information on major sites for ISF flow, building on our earlier study using 20 µl tracer injection (Mihaylov et al 2002). Five adult female Wistar rats were anaesthetised (I.P.) to reach surgical anaesthesia (details in Youdim et al. 2004: 2.7ml/kg of diluted anaesthetic solution (50% water :25% Hypnorm (fentanyl citrate 0.315mg/ml + fluanisone 10mg/ml Jansen): 25% Hypnovel (midazolam 5mg/ml, Roche)). 1µl artificial CSF containing tracers Evans blue-albumin (EBA, 50mg/ml) and [^{3}H] inulin (4µCi/µl) was stereotactically injected in the grey-white matter junction of the corpus callosum and caudate putamen, 3.3mm deep over a period of 2 minutes. Three hours later the rats were humanely killed and their brains removed, frozen, sectioned (40 µm) and prepared for image analysis (optical density for EBA, autoradiography for [^{3}H] inulin).

Modified MATLAB software was used to plot the concentration of both tracers (from optic density) as a function of distance from the point of injection (POI), in three planes (medial-lateral, dorsal-ventral, anterior-posterior). The plots were compared to theoretical curves as predicted for diffusion (Nicholson, 1984).

For both tracers, deviation from the theoretical curves was evident, with spread to greater distances than predicted by diffusion, consistent with some movement by bulk flow of ISF. Asymmetries in distribution profiles were also observed, suggesting the existence of vectorial flow. Superimposition of tracer images onto the corresponding rat brain histology atlas (Paxinos and Watson, 1986) showed preferential movement of tracers along the corpus callosum (white matter tracts) with extensions into the overlying cortex, and some spread into the caudate putamen (grey matter). In one brain, a different pattern was seen, suggesting spread particularly in the perivascular space around major vessels (middle cerebral artery and branches). The pattern appeared to be critically dependent on injection site.

Bulk flow of ISF has implications for non-synaptic communication, for clearance of toxic metabolites such as β-amyloid in Alzheimer's disease, for migration of tumour and stem cells, and for drug delivery to the brain (Abbott, 2004).

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Ability of phenolic compounds to protect the blood-brain barrier against oxidative stress

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The endothelial cells lining cerebral capillaries form the blood-brain barrier (BBB), and help to protect the brain from potentially damaging blood or brain derived substances (Abbott, 2002). Oxidative stress (OS) to endothelium as well as neurons may play a causative or exacerbating role in several neuropathologies including stroke (Gilgun-Sherki et al., 2001). Certain plant-derived phenolic compounds including flavonoids have the potential to protect neurones and endothelial cells from OS (Youdim et al., 2002), but there has been little work on their effects on the BBB. In this study we examined gallic acid lauryl ester (GAL) and two phenolic acids produced by the colonic microflora, 3-hydroxyphenylacetic acid (HPA) and 3-(3-hydroxyphenyl)-propionic acid (HPP) for their ability to protect brain endothelial cells from OS, using an immortalised mouse endothelial cell line bEND5.

bEND5 cells (from ECACC) were seeded at 1.0 X 10^4 cells/200 µl medium per well in 96-well plates and grown to confluence. The cells were incubated with 0 to 1600µM H_2O_2 for 60 min to generate OS. Cytotoxicity/mitochondrial function was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. H_2O_2 caused a concentration-dependent induction of cytotoxicity, with over 50% cells viable at 200µM H_2O_2 (64.51±3.77% (mean ± s.e.m., n = 21). To test protective effects, different concentrations (2µM-200µM) of each phenolic compound were given either alone, with 200µM H_2O_2 (co-incubation) or before H_2O_2 (pre-incubation) for 60 min, followed by MTT assay.

GAL decreased MTT reduction in a concentration-dependent manner, while HPA significantly increased MTT reduction ($P<0.05$, Student's t test). HPP had no significant effect. In co-incubation studies, all three compounds increased MTT reduction in a concentration-dependent manner, with HPA showing polyphasic effects ($P<0.05$). Pre-incubation with GAL resulted in a concentration-dependent decrease in MTT reduction, while HPA and HPP showed polyphasic protective effects ($P<0.05$).

The results show that HPA and HPP significantly protected the cells from H_2O_2-induced OS, consistent with action by scavenging reactive oxygen species (ROS) and activating protective mechanisms. GAL was protective in co-treatment, but was toxic to cells when applied alone and when given as pre-treatment. These findings suggest that selected phenolic compounds have valuable protective potential against OS at the BBB, while GAL may be more suitable as an anti-tumour agent.

Abbott, N.J. (2002) J. Anat. 200, 629-638

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