Validation of astrocytic cAMP signalling to study therapeutic targets for Alzheimer's disease

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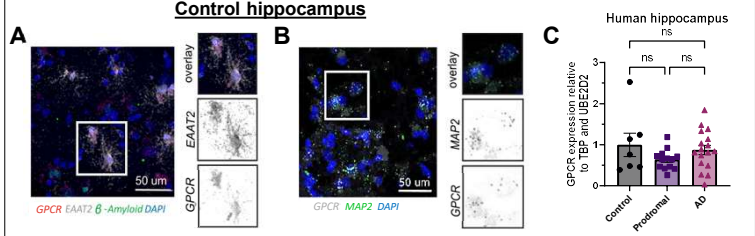
1. Introduction

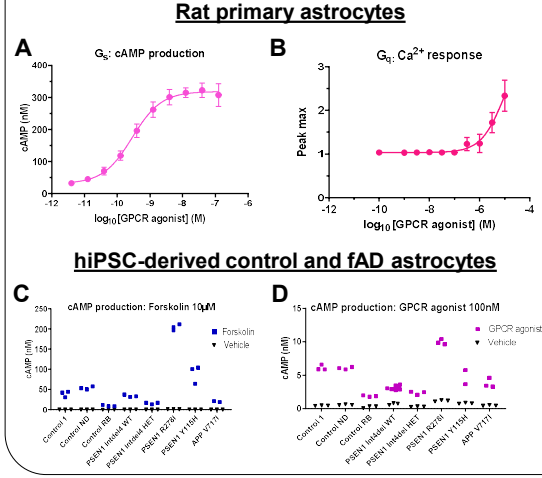
Astrocytes play a fundamental role in pathological processes associated with neurodegenerative diseases, including neuroinflammation, impaired glutamate uptake, reduced neurotrophic support and defective metabolism. Activation of cAMP signalling in astrocytes elevates glycolytic rate, increases glutamate transporter and neurotrophic factor expression, and suppresses the immune response. Molecules that modulate astrocytic cAMP signalling are therefore potential therapeutic targets for neurodegenerative diseases such as Alzheimer's disease (AD). To support the identification and validation of astrocyte-centric targets, we have optimised a suite of *in vitro* assays including cAMP, Ca^{2+}, and metabolic assays, multi-electrode array (MEA) and RNA-seq in rodent, human foetal and hiPSC-derived control and familial AD (fAD) astrocyte models. We have tested the adenylyl cyclase activator forskolin and a GPCR agonist in these assays to validate and increase our confidence in astrocytic cAMP signalling as an AD drug target.

2. Levels of a G_s-coupled GPCR expressed by human astrocytes and neurons are unaltered in AD



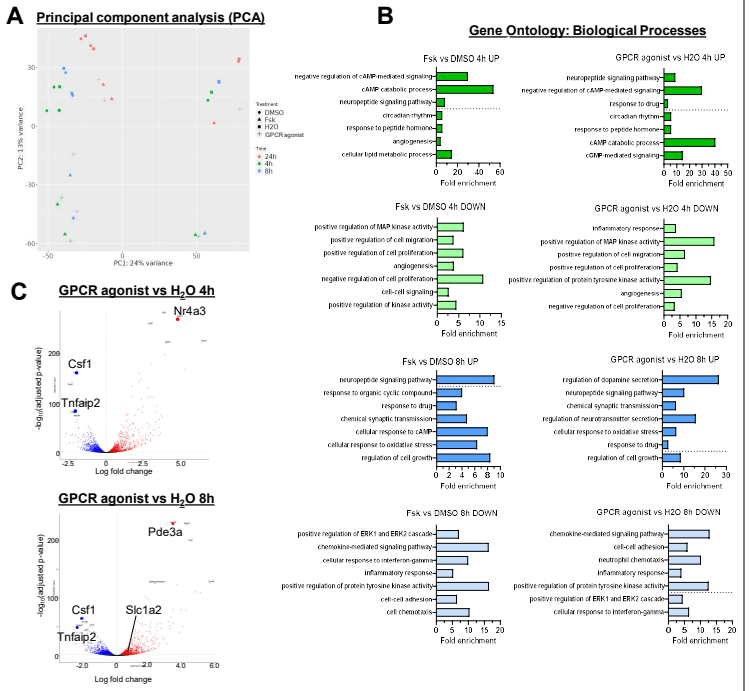
GPCR RNA expression in control and AD hippocampus. A-B) RNAscope analysis demonstrating GPCR co-localisation with EAAT2 (astrocytic marker; (A)) and MAP2 (neuronal marker; (B)) in human control hippocampus. C) qPCR analysis of GPCR RNA expression relative to TBP and UBE2D2 in human hippocampus, n=7-17 biological replicates. qPCR data shown as mean ± SEM and analysed using one-way ANOVA, ns= non-significant.

3. Forskolin and a GPCR agonist induce cAMP activation in astrocytes *in vitro*



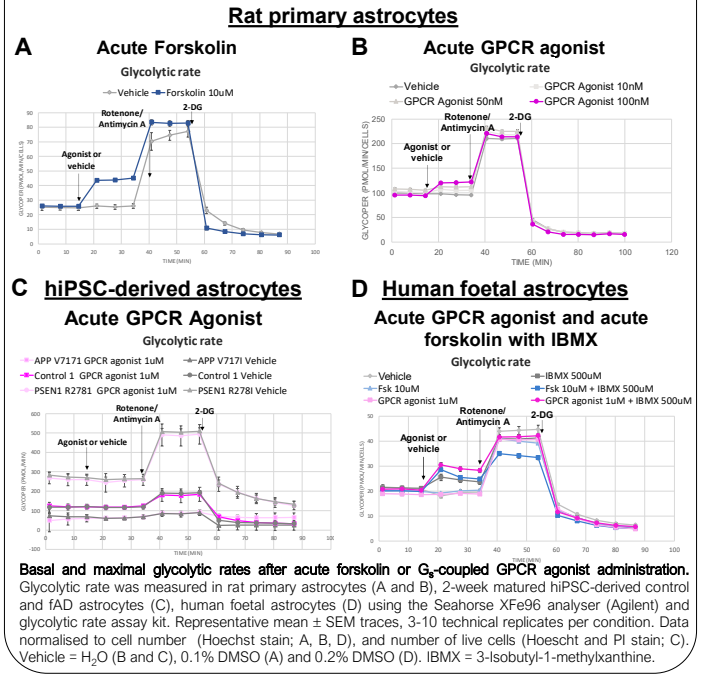
GPCR agonist induced cAMP and Ca^{2+} production in rat primary astrocytes and forskolin and GPCR agonist induced cAMP production in control and fAD hiPSC-derived astrocytes. (A), (C) and (D) cAMP accumulation was measured after a 1 hour agonist incubation using the cAMP G_s HiRange HTRF kit (Cisbio) in rat primary astrocytes (A), n=3 biological replicates, and 2 week-matured hiPSC-derived control and fAD astrocytes (C and D), n=3-6 technical replicates. (B) Maximum Ca^{2+} response (Peak max) after acute agonist addition was measured using the FLIPR Calcium 6 assay kit (Molecular devices) in rat primary astrocytes, n=2 biological replicates. Data presented as mean ± SEM (A and B). Vehicle = 0.1% DMSO (C and D).

4. cAMP activation in rodent primary astrocytes reduces the expression of inflammatory genes



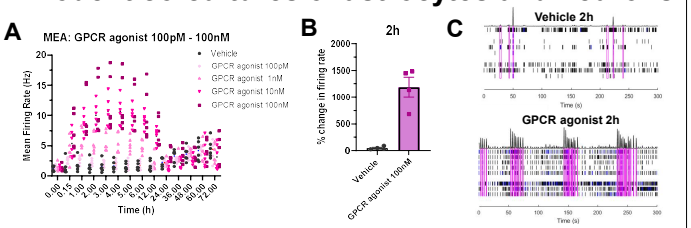
RNA-seq analysis of rat primary astrocytes treated with 10μM forskolin or 100nM G_s-coupled GPCR agonist for 4, 8 or 24h. (A) PCA analysis, (B) Gene Ontology (GO) analysis for biological processes carried out on the 200 significantly up-regulated genes (UP) and the 200 significantly down-regulated genes (DOWN), with the greatest $\log_2$ fold change at the 4h and 8h timepoints. Pathways below dashed line have a false–discovery rate (FDR)>0.05. (C) Volcano plots of significantly upregulated genes (red; FDR<0.05) and downregulated genes (blue; FDR<0.05) at 4h and 8h post-GPCR agonist treatment. (D) FPKM values of individual genes. N=3 biological replicates per condition.

5. cAMP activation increases glycolytic rate in rodent primary and human foetal astrocytes



Basal and maximal glycolytic rates after acute forskolin or G_s-coupled GPCR agonist administration. Glycolytic rate was measured in rat primary astrocytes (A and B), 2-week matured hiPSC-derived control and fAD astrocytes (C), human foetal astrocytes (D) using the Seahorse XFe96 analyser (Agilent) and glycolytic rate assay kit. Representative mean ± SEM traces, 3-10 technical replicates per condition. Data normalised to cell number (Hoechst stain; A, B, D), and number of live cells (Hoescht and PI stain; C). Vehicle = H_2O (B and C), 0.1% DMSO (A) and 0.2% DMSO (D). IBMX = 3-Isobutyl-1-methylxanthine.

6. A GPCR agonist enhances neuronal activity in rodent co-cultures of astrocytes and neurons



Neuronal activity in rat primary co-cultures of astrocytes and neurons treated with a GPCR agonist. (A) Agonist or vehicle was administered at DIV14 at 0.15h; mean firing rate was recorded for 15 minutes at the indicated timepoints using the Maestro Pro multielectrode array (MEA) system (Axion Biosystems), n=6 technical replicates. (B) Percentage change in firing rate vs baseline at 2h post-agonist or vehicle treatment, n=4 biological replicates. (C) Raster plot of neuronal activity in a single well 2 hours post-agonist or vehicle treatment. Vehicle = cell culture media.

7. Summary

We have demonstrated that RNA levels of a G_s-coupled GPCR are not altered in the AD brain. Both forskolin and the GPCR agonist elevate glycolytic rate in rat and human foetal astrocytes, suggesting that the mechanism is cAMP-mediated. Interestingly, the GPCR agonist enhances neuronal firing *in vitro* in neuron-astrocyte co-cultures. Finally, RNA-seq analysis identified cAMP pathway and inflammatory genes as major targets of both forskolin and GPCR agonist administration. Overall, our data suggest that activation of astrocytic cAMP signalling has exciting therapeutic potential as a treatment for AD.

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