Development of UPLC-MS/MS Method to Quantify Baseline Levels of Endocannabinoids in Mouse Brain

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Introduction

The endocannabinoid (eCB) system has been identified as an important neuromodulatory system, responsible for many physiological functions, such as pain, neuroprotection, cognitive function, and motor activity[1]. It is comprised of endogenous cannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), their respective metabolic enzymes, fatty-acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), and the cannabinoid type-1 (CB_1) and type-2 (CB_2) receptors[2]. Previous reports[3] have stated degradation of 2-AG after collection of brains, and during sample processing is prevalent, while AEA seems to be unchanged during this process. Our goal is to develop a sensitive bioanalytical method following FDA M10 guidelines, to quantify baseline levels of AEA and 2-AG in the brain of C57BL/6N mice and stabilize further metabolism of 2-AG during sample processing and storage.

Discussion

- Charcoal-stripped plasma was used as a surrogate matrix to ensure complete removal of endogenous AEA and 2-AG
- A range of 1-250 ng/mL was found to be linear, with a correlation value of >0.99 using $1/x^2$ weighing factor
- 50 mM Tris buffer with 50 nM NF1819 (MAGL inhibitor) prevented further metabolism of 2-AG while processing brain samples
- Equivalent concentrations of AEA in whole mouse (C57BL/6N) brain were 30.8-45.8 ng/g in males and 35.4-53.5 ng/g in females
- 2-AG baseline concentrations in male and female mouse brains (C57BL/6N) were 695.3-2344.2 ng/g and 709.5-1957.7 ng/g, respectively
- No significant sex differences were observed between baseline levels of AEA or 2-AG in mice

Methods

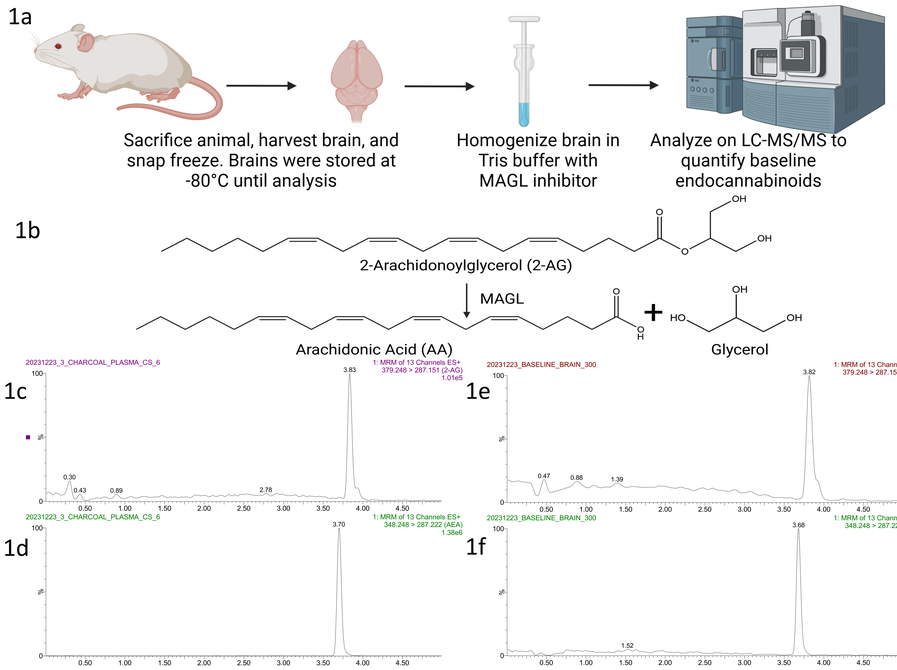

Figure 1a-f: a) schematic of study; b) metabolism of 2-AG by MAGL to AA and glycerol; c) calibration standard chromatogram of 2-AG and d) AEA in charcoal-stripped plasma surrogate matrix; e) chromatogram of baseline brain sample, 2-AG and f) AEA

Results

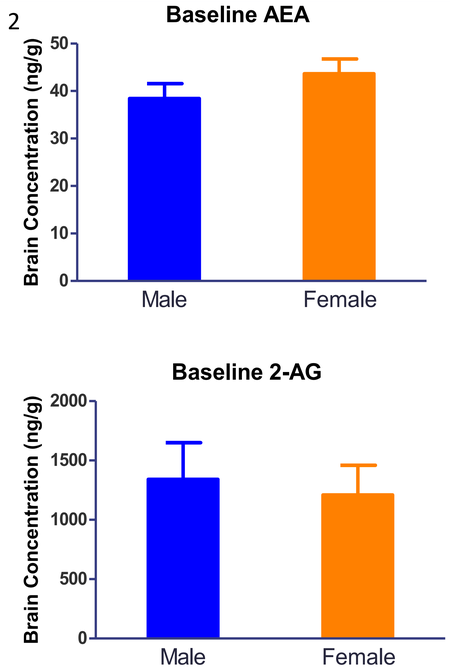

Figure 2: Baseline whole brain levels of AEA (top) and 2-AG (bottom) in male and female C57BL/6N wild-type mice.

Conclusions

This sensitive and selective bioanalytical method was successfully applied for the quantification of baseline brain levels of AEA and 2-AG in male and female C57BL/6N mice. These results could be used to understand the effect of different treatments on baseline endocannabinoid levels. By utilizing the MAGL inhibitor during sample processing, we were able to prevent degradation of 2-AG and achieve a linearity range required for the quantification of endogenous endocannabinoids in mouse brain homogenate.

References

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