A FLUORESCENCE-DERIVATISATION ASSAY FOR FATTY ACID AMIDE HYDROLASE ACTIVITY

Michael J. Garle, J. Stephen Clark* and Stephen P.H. Alexander, Institute of Neuroscience and School of Biomedical Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, ENGLAND *School of Chemistry, University of Nottingham, Nottingham NG7 2RD, ENGLAND.

Fatty acid amide hydrolase (FAAH) is a key enzyme in the regulation of endocannabinoid levels, along with monoacylglycerol lipase and the lysosomal enzyme *N*-acylethanolamine acid amidase (NAAA) (Rodríguez de Fonseca *et al.*, 2005). FAAH catalyses the hydrolysis of *N*-acylethanolamines (including anandamide), monoacylglycerols (including 2-arachidonoylglycerol) and primary amides (including oleamide). We have previously described an assay for FAAH, which was based on the estimation of the ammonia generated upon oleamide hydrolysis by the enzyme. A coupled enzyme system was used to determine ammonia generation, measuring the destruction of NADH associated with incorporation of ammonia into glutamine under the influence of glutamic acid dehydrogenase (De Bank *et al.*, 2005). Here, we describe an alternative FAAH assay based on measurement of accumulated ammonia using fluorescence derivatisation.

Membrane fragments (rich in FAAH activity) were prepared from brains and livers of male Wistar rats (150-250 g) as described previously (De Bank *et al.*, 2005). After incubation with oleamide (0-200 µM) for 30 min, the reaction was halted by addition of trichloroacetic acid to a final concentration of 1.6 % w/v. After centrifugation (5' at *ca.* 5000 g) to remove protein, ammonia production was determined by the fluorescent isoindole-1-sulfonate product following incubation with alkaline o-phthaldehyde and sodium hydrosulphite for 30 min (Mana and Spohn, 2001). Data were generated from at least four separate tissue preparations.

Kinetic values for FAAH activity in liver were calculated from mean concentration response curves with K_m and V_{max} values of 129 µM and 15 nmol.min.mg^{-1} protein, respectively, consistent with our previous observations (De Bank *et al.*, 2005). In preparations from brain, the enzyme had similar substrate affinity (K_m value of 179 µM), but capacity (V_{max}) was greatly reduced at 1.03 nmol.min.mg^{-1} protein.

Simultaneous incubation of rat liver preparations in the presence of 100 µM oleamide with the non-selective serine protease inhibitor phenylmethylsulfonylfluoride (200 µM) or the selective FAAH inhibitor URB597 (1 µM, Mor *et al.*, 2004) reduced FAAH activity from 7.45 ± 0.32 to 0.45 ± 0.15 and 0.39 ± 0.08 nmol.min.mg^{-1}, respectively. Analysis of increasing concentrations of URB597 and the selective NAAA inhibitor *N*-cyclohexanonecarbonylpentadecylamine (Tsuboi *et al.*, 2004) revealed a differential inhibitory effect on FAAH activity with calculated *p*IC_{50} values of 6.6 ± 0.1 and <5.3, respectively.

In summary, therefore, we describe here a novel fluorescence-based assay for FAAH activity suitable for application in medium throughput screening.

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