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The application of a fluorogenic substrate for recombinant αβ hydrolase 6 (ABHD6) activity

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Background: Levels of monoacylglycerols (MAGs) depend on synthesis (via diacylglycerol lipases), transformation (via, for example, cyclooxygenase-2) and hydrolysis. Monoacylglycerol lipase (MAGL) is considered to be responsible for the bulk of 2-arachidonoylglycerol and 2-oleoylglycerol hydrolysis in the brain, alongside 'minority' enzymes, such as ABHD6 and ABHD12[1]. While much effort has been directed towards the study of MAGL, there are many fewer investigations of ABHD6 activity. We have recently defined 4-methylumbelliferylheptanoate (MUH) as a fluorogenic substrate for ABHD6 (ICRS 2017). We have extended these studies to characterise the use of this substrate for inhibitor screening.

Methods: HEK293 cells, transiently transfected with human ABHD6, were harvested 48 hours after transfection and the membrane fraction isolated by differential centrifugation. MUH hydrolysis was assessed in Tris EDTA (50:1 mM, pH 7.4) buffer in 96-well microtitre plates at 37 °C for 60 min, monitoring 4-methylumbelliferone production (ex 355, em 460 nm). Data were generated using at least five separate preparations of transfected cells.

Results: ABHD6-HEK293 cell particulate preparations hydrolysed MUH with an affinity of 29 ± 6 µM. Using 50 µM MUH as substrate, WWL70 (previously described as a selective ABHD6 inhibitor[2]) caused a concentration-dependent inhibition (pIC_{50} value 7.3 ± 0.05), with a small residual activity (6 ± 1 % control). Methylarachidonoylfluorophosphonate caused a potent, complete inhibition (pIC_{50} value of 8.0 ± 0.01). Surprisingly, the reportedly ABHD6-selective inhibitor WWL123[3] only produced an incomplete inhibition (45 ±2 % control, pIC_{50} 6.3 ± 0.1). Additionally, JJKK048, reported to be a selective inhibitor of MAG lipase[4], also produced a complete inhibition, albeit at lower potency (7.1 ± 0.06). Using 1 µM WWL70 as a comparison allowed calculation of a Z' factor of 0.42. MUH hydrolysis was inhibited in the presence of 100 µM 2AG (68 ± 4 % control), 2OG (84 ± 2) or NAGly (82 ±2), but not AEA, 1OG or 2PG. Blind screening of a range of drugs at 10 µM identified that most were ineffective, with the exception of orlistat (31 ± 13 % control).

Conclusions: MUH hydrolysis represents a useful method for high-throughput screening of ABHD6 modulators.

1. Blankman et al. (2007). Chem Biol PMID:18096503

2. Li et al. (2007). J Am Chem Soc PMID:17629278

3. Bachovchin et al. (2010). Proc Natl Acad Sci PMID:21084632

4. Aaltonen et al. (2013). Chem Biol PMID:23521796

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