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A novel fluorescence-based assay for fatty acid amide hydrolase activity based on quantification of ethanolamine production

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The *N*-acylethanolamide anandamide (AEA) acts as an agonist at the G protein-coupled receptors CB_1 and CB_2 cannabinoid receptors, as well as at the ligand-gated ion channel TRPV1 and the nuclear hormone receptor peroxisome proliferator-activated receptor γ (1). Levels of anandamide are regulated by the hydrolytic enzyme, fatty acid amide hydrolase (FAAH), which also regulates levels of a number of structurally-related endogenous *N*-acylethanolamides, including *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA), which also act at multiple receptors (1). The most common assay of FAAH activity involves the use of *N*-arachidonoyl-[^{3}H]-ethanolamine (3), although we (and others) have described spectrophotometric assays based around oleamide hydrolysis and ammonia quantification (2).

In this report, we describe a novel versatile FAAH activity, which can be applied to measure hydrolysis of any *N*-acylethanolamine.

Naphthalene-2,3-dicarbaldehyde was dissolved in acetonitrile to 1 mg/mL and then diluted 1:5 in 0.2 M borate buffer, pH 11.0 (4). Increasing concentrations of ethanolamine were allowed to react at room temperature, before samples were assessed for fluorescence with excitation at 420 nm and emission at 480 nm. Ammonia failed to generate a fluorescent product under these conditions. Optimisation of the ethanolamine quantification included an assessment of the pH of the borate buffer, the latency and stability of development of the fluorescent adduct, a comparison of trichloroacetic acid and perchloric acid as stopping reagent. Fluorescence increased over 30 min of incubation with ethanolamine and then maintained a stable plateau for a further hour. Blanks in the presence of trichloroacetic acid were modestly lower than those with perchloric acid.

Rat liver microsomes (50 µg protein/mL) were utilised as a source of FAAH activity, halting incubations with 10 % (w/v) trichloroacetic acid, before diluting samples of the resultant supernatant layer 3-fold in the NDC/acetonitrile/borate mix. Hydrolysis of OEA, as an example substrate, was linear for up to 30 min in the presence of rat liver microsomes. Rat liver microsomes hydrolysed four endogenous *N*-acylethanolamines with the kinetic characteristics described in the table (n=4).

Table: Kinetics of *N*-acylethanolamine hydrolysis by rat liver FAAH activity (n=6)

Substrate	K_m (µM)	V_{max} (nmol/min/mg protein)
PEA (C16:0)	2.8 ± 0.4	7.8 ± 0.8
N-Stearoylethanolamine	7.1 ± 1.3	4.7 ± 0.5

(C18:0)		
OEA (C18:1)	38 ± 10	53 ± 12
Anandamide (C20:4)	33 ± 3	40 ± 2

In summary, we describe a novel, versatile assay for FAAH activity which allows the hydrolysis of multiple *N*-acylethanolamines to be quantified.

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