Supplemental Materials

Endocannabinoids – Anandamide and 2- Arachidonoylglycerol are Substrates for Human Cytochrome P450 2J2 Epoxygenase

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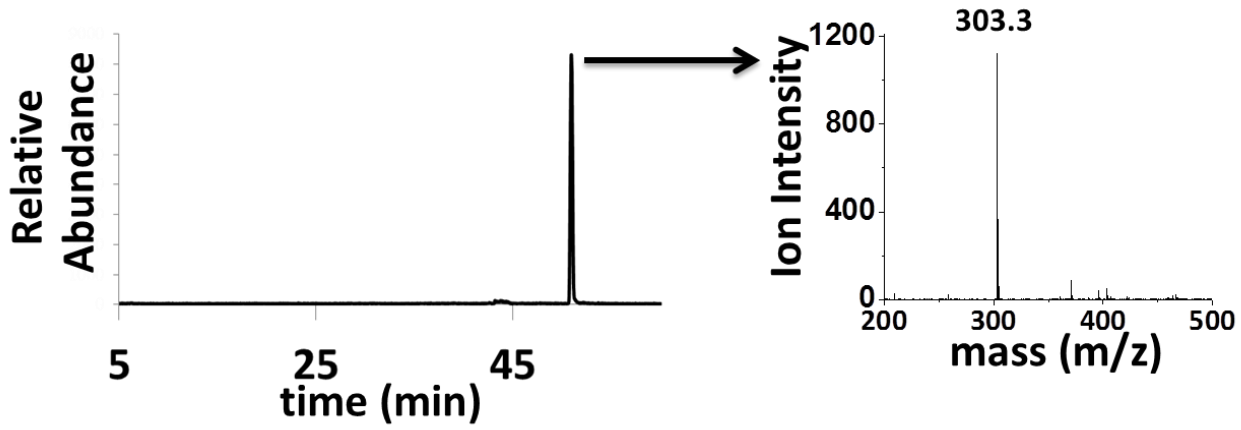
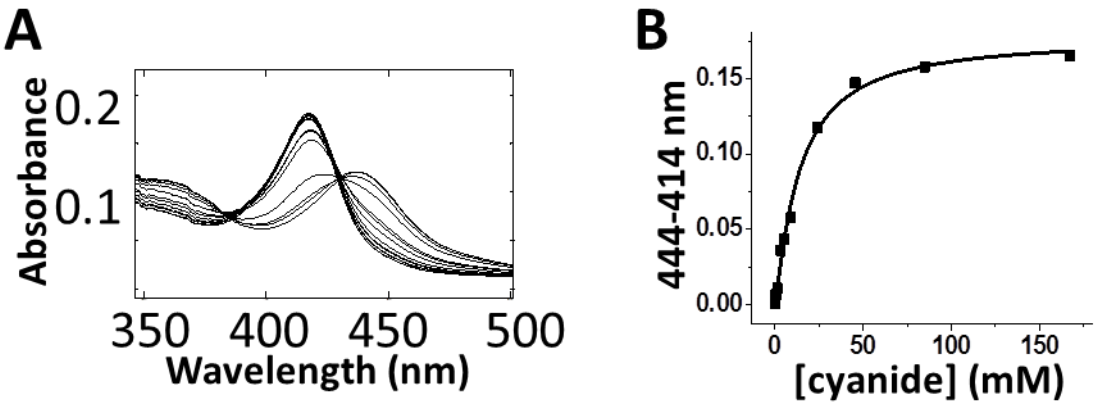
	Steady State Cyanide Binding	Stopped Flow Cyanide Binding Kinetics		
	Cyanide (K_s)	**Fast-Phase Rate (s^{-1})**	**Slow Phase Rate (s^{-1})**	**Fast-Phase (%)**
Substrate Free	3.1 ± 0.2	5.6 ± 0.1	0.6 ± .06	82.6 ± 2.08
AA (10 μM)	NT	5.5 ± 0.4	0.8 ± 0.2	76.6 ± 2.0
AA (20 μM)	NT	5.4 ± 0.3	1.1 ± 0.1*	74.2 ± 2.7
AA (40 μM)	7.7 ± 1.1	4.9 ± 0.2	1.19 ± 0.1**	69.3 ± 3.4
AEA (10 μM)	NT	5.1 ± 0.1	0.7 ± .04	79.3 ± 1.6
AEA (20 μM)	NT	5.0 ± 0.1	0.9 ± 0.01*	78 ± 0.3
AEA (40 μM)	5.2 ± 1.4	4.1 ± 0.1*	1.1 ± 0.02**	71 ± 0.3
2-AG (10 μM)	NT	5.2 ± 0.3	0.8 ± 0.1	77.6 ± 4.1
2-AG (20 μM)	NT	5.1 ± 0.1	0.9 ± 0.07*	76.8 ± 1.6
2-AG (40 μM)	4.3 ± 0.2	5.1 ± 0.01	1.0 ± .01**	71.5 ± 0.4
Ebastine (10 μM)	NT	4.2 ± 0.1*	0.46 ± 0.01*	49.5 ± 0.1
Ebastine (20 μM)	NT	4.3 ± 0.08*	0.46 ± .01*	37.4 ± 0.29
Ebastine (40 μM)	13.3 ± 0.2	3.4 ± 0.04***	0.41 ± .001**	33.6 ± 0.42
Palmitate (40 μM)	NT	5.5 ± 0.04	0.6 ± .02	80 ± 0.1
MSPPOH (10 μM)	16.1 ± 0.51	4.1 ± .04	0.49 ± .016	64.6 ± 1.82

NT = not tested

Supplemental Table 1. *Summary of cyanide binding in the presence of fatty acids and ebastine.* Substrate concentrations were compared to the substrate free sample and significance was attained when $p < 0.05$, $p < 0.01$ and $p < 0.001$ and were denoted by *, **, and ***, respectively

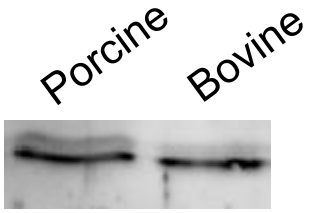


Supplemental Figure 1. *Incubations of CYP2C8 supersomes with 2-AG*. Incubations containing CYP2C8 and 2-AG were performed as described in the materials and methods. Product analysis revealed the presence of arachidonic acid in the reaction but did not produce detectable levels of EET-G or EETs.



Supplemental Figure 2. *Steady-state cyanide binding to substrate-free and substrate bound CYP2J2-ND.* (A) The UV-Vis spectra of an equilibrium binding titration of cyanide to CYP2J2-ND. The binding of cyanide red shifted the Soret band. (B) The change in absorbance (ΔAbs) from 444 nm to 414 nm was calculated for each titration and plotted against the corresponding cyanide concentration. Data were fit to the single binding isotherm by using Origin Pro 8.6. The cyanide K_s (μM) of substrate-free and 40 μM substrate bound were calculated for each substrate. The apparent binding constant (K_s) of the steady state cyanide binding to the active site of substrate free CYP2J2-ND was calculated at 3.1 ± 0.2 mM. The presence of 40 μM of either AA or AEA modestly increased the Ks of cyanide binding to 7.7 ± 1.1 mM and 5.2 ± 1.4 mM, respectively. The presence of 2-AG only slightly shifted the K_s of cyanide binding to 4.3 ± 0.2 mM. When the system was incubated with 40 μM of ebastine, the K_s of cyanide binding more than tripled with values of 13.3 ± 0.2 mM (Supplemental Table 1). Notably, these results were inversely proportional to the equilibrium binding constants calculated from spectral binding assays. This suggests that a tighter substrate binding blocks cyanide access to the active site.

Supplemental Figure 3. *Multiple sequence alignment of human, bovine and porcine CYP2J2 sequences.* Inter-species alignments of CYP2J2 from porcine, bovine and calculated an 80% homology for comparison of porcine to bovine, 77% homology for porcine to human and a 75% homology for bovine to human. The single fully conserved residue for each of all of the species is denoted by (*). The majority of other residues polarity was maintained as represented by **(:)** for the conservation of strong groups and (.) for conservation of weak groups.



Supplemental Figure 4. *CYP2J2 immunoblotting in bovine and porcine microsomes using a polyclonal rabbit IgG antibody for human CYP2J2.* The inter-species cross-reactivity with the anti-body raised against the human CYP2J2 was examined. From left to right, lane 1 contains 100 ug of pig heart microsomes, lane 2 contains 100 ug of cow heart microsomes.