Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol17Issue1abst021P.pdf

Identification of medium chain N-acyl glycines as GPR132 (G2A) receptor agonists

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Introduction*:* The N-acyl amides are a family of endogenous signalling lipid molecules typically consisting of an acyl chain conjugated to a biological amine (which may be dopamine, an amino acid, etc). The prototypical member of this family, N-arachidonoyl ethanolamine, exerts biological activity through the cannabinoid receptors [1]. We show that the activity of this family of molecule extends to the orphan GPR132 (G2A) receptor, by demonstrating agonist activity of medium chain N-acyl glycines.

Method: Rat Basophilic Leukemia (RBL) cells stably expressing human G2A(a) were loaded with Fura-2 AM and microfluorimetry experiments performed to measure agonist-induced calcium mobilisation. Data was analysed using unpaired students T-test to compare RBL-G2A with RBL parental. For yeast experiments, human, rat, mouse, and zebrafish G2A receptors were stably integrated into modified yeast strains containing yeast-human G-protein chimeras. Enzyme mediated hydrolysis of fluorescein di-β-D-glucopyranoside was used to measure agonist-dependent yeast growth [2]. The DiscoverX PathHunter assay was used to measure agonist-induced βarrestin-2 association with human and mouse G2A.

Result: In the calcium microflourimetry experiments N-linoleoyl glycine (NLG) had equal agonist potency as the reported G2A endogenous agonist 9-hydroxyoctadecadienoic acid (9-HODE) (Figure 1)[3]. In yeast and βarrestin-2 association assays all G2A species orthologues tested were sensitive to medium chain N acyl-glycines (Table 1 and 2). N-palmitoyl glycine had higher potency than 9-HODE in the yeast assay, whereas 9-HODE was more potent than any medium chain N-acyl glycine tested in the βarrestin-2 association assay. Finally, we used a G2A antagonist to demonstrate inhibition of G2A agonism by N-acyl glycines on human G2A(a) (Figure 2).

Conclusion: We identify medium chain N-acyl glycines as agonists of the G2A receptor.

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[2] Dowell, S. J., & Brown, A. J. (2009). *Methods Mol Biol, 552*, 213-229. doi:10.1007/978-1-60327-317-6_15

[3] Obinata, H. et al. (2005). *J Biol Chem, 280*(49), 40676-40683. doi:10.1074/jbc.M507787200

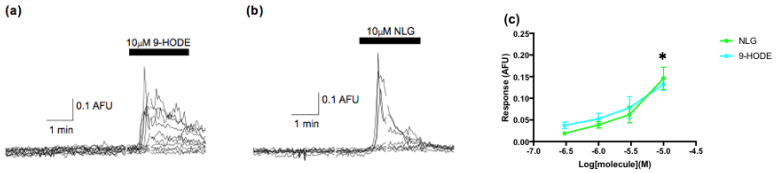

Figure 1 Calcium transients taken from microfluorimetry experiments showing effect of (a) 9-HODE and (b) NLG on a field of RBL-G2A cells. Traces represent changes in fura-2 fluorescence ratio for individual cells. (c) Concentration-response relationship between 9-HODE and NLG on RBL-G2A cells. Data are mean peak responses ± SEM of 30 cells derived from three independent experiments. *=sig. diff. compared to un-transfected RBL cells (unpaired t-test P<0.05, 4 df, n=3).

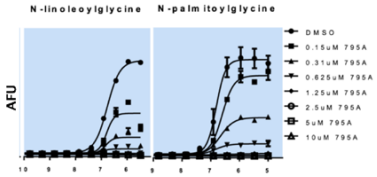

Figure 2. Antagonism of N-acyl glycines on human G2A(a) by synthetic antagonist 795A in yeast assay. Data expressed as mean ± SD of four replicates.

	G2A species orthologue			
N-Acyl Glycine	Human G2A(a)		Mouse G2A	
	pEC50	***Emax***	***pEC50***	***Emax***
N-palmitoyl glycine	5.37±0.58 (n=4)	2.53±1.02	n/a (n=3)	n/a
N-linoleoyl glycine	5.08±0.29 (n=6)	4.83±1.72	5.17±0.23 (n=5)	2.7±0.87
N-oleoyl glycine	5.53±0.07 (n=4)	3.69±0.32	n/a (n=5)	n/a
N-stearoyl glycine	5.45±0.08 (n=4)	2.4±0.18	n/a (n=4)	n/a
9-HODE	5.34±0.13 (n=7)	13.96±2.67	5.33±0.21 (n=6)	5.22±1.24

Table 2. Summary of pEC50 ± SEM and Emax (fold of basal) ± SEM values for N-acyl glycines active on G2A species orthologues in DiscoverX PathHunter ß-arrestin assay.

	G2A species orthologue											
N-Acyl Glycine	Human G2A(a)		Human G2A(b)		Mouse G2A		Rat G2A		Zebrafish G2A(a)		Zebrafish G2A(b)	
	pEC50	***Emax***	***pEC50***	***Emax***	***pEC50***	***Emax***	***pEC50***	***Emax***	***pEC50***	***Emax***	***pEC50***	***Emax***
N-palmitoyl glycine	6.27±0.05 (n=7)	16.25±0.66	6.18±0.1 (n=5)	13.75±1.05	6.51±0.25 (n=5)	1.44±0.07	6.9±0.07 (n=7)	1.29±0.02	6.13±0.07 (n=4)	1.91±0.03	6.92±0.06 (n=4)	1.37±0.01
N-linoleoyl glycine	5.81±0.06 (n=14)	15.33±0.98	5.81±0.08 (n=3)	9.8±0.82	6.17±0.16 (n=12)	1.22±0.03	6.11±0.08 (n=14)	1.51±0.05	.-.	.-.	.-.	.-.
N-oleoyl glycine	5.23±0.05 (n=4)	11.74±0.59	5.41±0.08 (n=3)	5.55±0.38	5.6±1.66 (n=4)	1.48±0.8	5.95±0.07 (n=4)	1.34±0.03	5.69±0.05 (n=4)	1.58±0.02	6.15±0.1 (n=4)	1.46±0.03
N-stearoyl glycine	5.15±0.03 (n=4)	10.77±0.36	n/a (n=3)	n/a	5.65±0.42 (n=4)	1.46±0.18	5.72±0.11 (n=4)	1.49±0.05	5.47±0.11 (n=4)	1.68±0.05	6.2±0.04 (n=4)	1.6±0.02
9-HODE	5.86±0.04 (n=4)	18.23±0.85	5.55±0.15 (n=3)	21.14±3.62	n/a	n/a	6.26±0.07 (n=3)	1.7±0.04	.-.	.-.	.-.	.-.

Table 1. Summary of pEC50±SEM and Emax (fold of basal) ± SEM values for N-acyl glycines active on G2A species orthologues in yeast assay. n/a = not active, .-. = data not available.