THE EFFECTS OF PHARMACOLOGICAL BLOCKADE OF PPARs ON FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR, FEAR-CONDITIONED ANALGESIA AND CONDITIONED FEAR IN THE PRESENCE OF NOCICEPTIVE TONE IN RATS

Jessica C. Gaspar[1,3,4], Bright Okine[1,3,4], Álvaro Llorente-Berzal[1,3,4], Orla Burke[1], David Dinneen[1], Michelle Roche[2,3,4], David P. Finn[1,3,4]

[1]Pharmacology and Therapeutics, [2]Physiology, School of Medicine, [3]Galway Neuroscience Centre and [4]Centre for Pain Research, NCBES, National University of Ireland, Galway, Ireland

Introduction

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors and exist in three isoforms: PPARα, PPARβ/δ and PPARγ[1].

PPARs are targets for endocannabinoids and *N*-acylethanolamines.

There is evidence for PPAR involvement in pain[2] and cognition[3], however, their role in pain-fear interactions is unknown.

The amygdala, particularly the basolateral amygdala (BLA), plays a key role in pain, conditioned fear and fear-conditioned analgesia (FCA)[4]. All three PPAR isoforms are expressed in the amygdala[5].

The experimental procedures were approved by the Animal Care and Research Ethics Committee, National University of Ireland Galway, and the work was carried out under license from the Health Products Regulatory Authority in the Republic of Ireland and in accordance with EU Directive 2010/63.

Aim

Investigate the effects of systemic and intra-BLA administration of PPARα, PPARβ/δ and PPARγ antagonists on formalin-evoked nociceptive behaviour, FCA, and conditioned fear in the presence of nociceptive tone in rats.

References

[1]Blanquart, C., Barbier, O., Fruchart, J.C., Staels, B., and Glineur, C. (2003). *Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation*. J. Steroid Biochem. Mol. Biol. *85*: 267–73.

[2]LoVerme J, Russo R, La Rana G, Fu J, Farthing J, Mattace-Raso G, Meli R, Hohmann A, Calignano A, Piomelli D., *Rapid broad-spectrum analgesia through activation of peroxisome proliferator-activated receptor-alpha.* The Journal of Pharmacology and experimental Therapeutics (2006) 319: 1051-61

[3]Petrosino S, Ahmad A, Marcolongo G, Esposito E, Allarà M, Verde R, Cuzzocrea S, Di Marzo V., *Diacerein is a potent and selective inhibitor of palmitoylethanolamide inactivation with analgesic activity in a rat model of acute inflammatory pain.* Pharmacological Research (2015) 91: 9-14.

[4]Izquierdo, I., Furini, C.R.G., and Myskiw, J.C. (2016). *Fear Memory*. Physiol. Rev. *96*:.

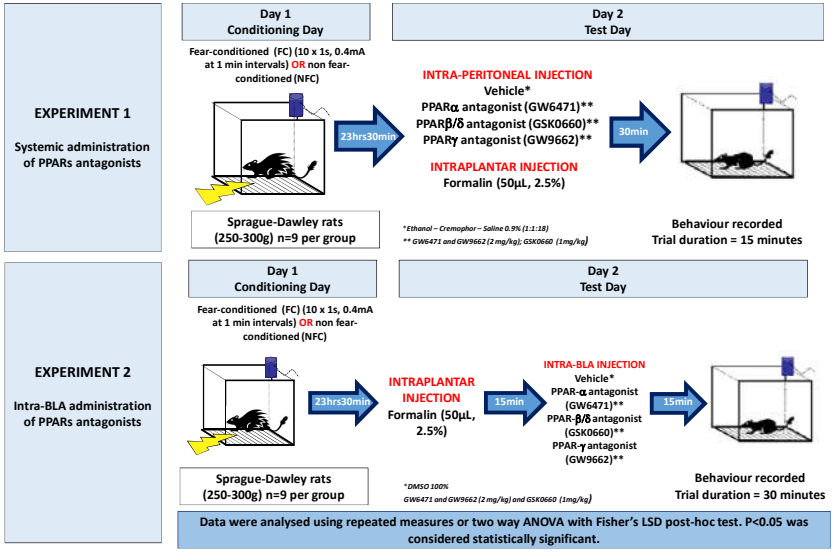
[5]Warden, A., Truitt, J., Merriman, M., Ponomareva, O., Jameson, K., Ferguson, L.B., et al. (2016). *Localization of PPAR isotypes in the adult mouse and human brain*. Sci. Rep. *6*: 27618.

Acknowledgements

Funding from CNPq, through the program "Science without borders" (207530/2014-9), Travel Bursaries from ICRS 2017, Neuroscience Ireland and British Pharmacological Society are gratefully acknowledged.

No conflit-of-interests

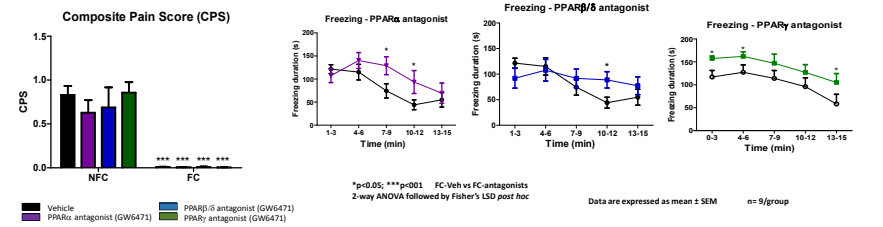
Methods



Data were analysed using repeated measures or two way ANOVA with Fisher's LSD post-hoc test. P<0.05 was considered statistically significant.

Results – Experiment 1

Systemic administration of PPARα , PPARβ/δ and PPARγ antagonists had no effect on formalin-evoked nociceptive tone or FCA, but potentiated contextually induced freezing in the presence of nociceptive tone

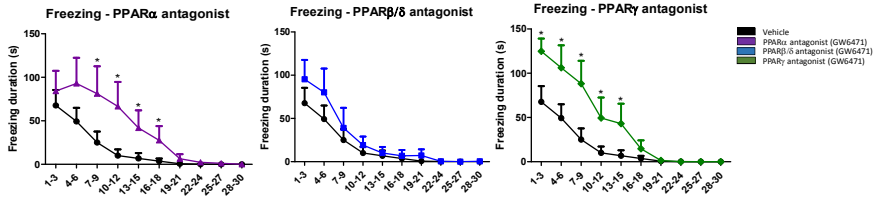


*p<0.05; ***p<001 FC-Veh vs FC-antagonists
2-way ANOVA followed by Fisher's LSD *post hoc*

Data are expressed as mean ± SEM n= 9/group

Results – Experiment 2

Intra-BLA administration of PPARα or PPARγ antagonists potentiated contextually induced freezing in the presence of formalin-evoked nociceptive tone



*p<0.05; FC-Veh vs FC-antagonists
2-way ANOVA followed by Fisher's LSD *post hoc*

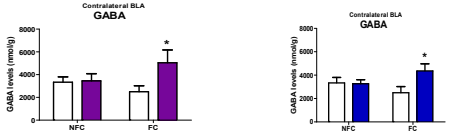
Data are expressed as mean ± SEM
n= 8-9/group

Results – Experiment 1

PPAR antagonist-induced potentiation of conditioned fear-related behaviour was associated with increased levels of GABA in the contralateral BLA, and decreased levels of dopamine and increased levels of PEA in the ipsilateral CeA.

Basolateral Amygdala (BLA)

		Ipsilateral								Contralateral							
		Neurotransmitters (nmol/g)				ECs/NAEs (nmol/g)				Neurotransmitters (nmol/g)				ECs/NAEs (nmol/g)			
		GABA	Glu	Dopa	5-HT	2-AG	AEA	PEA	OEA	GABA	Glu	Dopa	5-HT	2-AG	AEA	PEA	OEA
NFC	Vehicle	2360.12 ± 361.14	6209.92 ± 1050.11	202.74± 24.93	2445 ± 0.024	4.219 ± 1.065	0.024 ± 0.007	0.399 ± 0.11	0.566 ± 0.14	3336.27 ± 464.11	5759.84 ± 1002.01	193.70 ± 30.07	2.452 ± 0.274	1.965 ± 0.651	0.0162 ± 0.0045	0.435 ± 0.057	0.465 ± 0.08
	PPARα	3472.92 ± 431.01	13333.21 ± 4146.34	202.76 ± 32.99	2.725 ± 0.525	3.304 ± 0.987	0.026 ± 0.003	0.363 ± 0.05	0.475 ± 0.04	3452.92 ± 633.40	13166.46 ± 3269.74	243.47 ± 64.96	2.114 ± 0.171	2.136 ± 0.873	0.036 ± 0.008	0.328 ± 0.052	0.414 ± 0.06
	PPARβ/δ	2836.75 ± 342.75	8750.62 ± 2112.93	223.45 ± 55.26	2.587 ± 0.512	3.180 ± 0.753	0.027 ± 0.005	0.421 ± 0.06	0.514 ± 0.06	3265.90 ± 344.12	6900.19 ± 1855.41	233.133 ± 55.57	2.488 ± 0.241	3.822 ± 0.901	0.038 ± 0.012	0.592 ± 0.187	0.409 ± 0.08
	PPARγ	2370.80 ± 286.42	2852.121 ± 724.76	48.16 ±7.31	0.017	1.732 ± 0.507	0.018 ± 0.004	2.039 ± 0.44	0.192 ± 0.02	1960.30 ± 270.13	3650.08 ± 672.99	18.601 ± 3.166	0.082 ± 0.010	1.380 ± 0.193	0.014 ± 0.003	0.902 ± 0.243	0.174 ± 0.03
FC	Vehicle	2468.69 ± 558.42	**6398.06 ±1523.29#**	129.36 ± 28.25	2.042 ± 0.427	2.886 ± 0.594	0.15734 ± 0.0033	0.435 ± 0.06	0.507 ±0.08	2495.82 ± 525.32	5552.26 ± 1213.58	171.008 ± 30.98	2.078 ± 0.435	0.8389 ± 0.17	**0.0063 ± 0.001***	0.437 ± 0.057	0.544 ± 0.05
	PPARα	**3984.37 ± 664.77***	7905.93 ± 2096.28	221.77 ± 52.07	2.427 ± 0.704	3.485 ± 0.930	0.032 ± 0.008	0.328 ± 0.05	0.454 ± 0.08	**5817.00 ± 1261.38***	7956.71 ± 849.25	226.794 ± 60.455	1.773 ± 0.270	1.996 ± 0.709	**0.0333 ± 0.0046***	0.303 ± 0.052	0.457 ± 0.05
	PPARβ/δ	3296.54 ± 427.76	6231.73 ± 1090.34	206.87 ± 30.34	2.541 ± 0.284	1.624 ± 0.392	0.025 ± 0.005	0.803 ± 0.38	0.504 ± 0.12	**7060.15 ± 2748.75***	**10022.73 ± 1701.72***	228.622 ± 34.551	2.194 ± 0.554	4.442 ± 1.077	0.043 ± 0.014	0.454 ± 0.077	0.569 ± 0.11
	PPARγ	1810.88 ± 229.60	2307.83 ± 566.38	29.61 ± 4.23	0.051 ± 0.009	1.557 ± 0.292	0.0076 ±0.0017	1.332 ± 0.31	0.169 ± 0.03	2319.53 ± 434.11	4154.69 ± 680.87	23.333 ± 7.872	0.081 ± 0.024	**1.7973 ± 0.49***	0.016 ± 0.004	7.979 ± ±0.538	0.126 ± 0.03

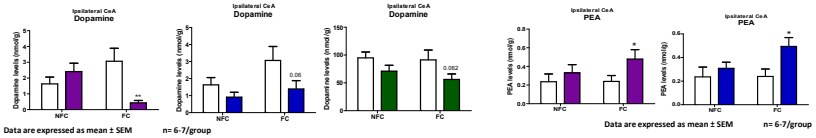


*p<0.05; **p=001 FC-Veh vs FC-antagonists
2-way ANOVA followed by Fisher's LSD *post hoc*

Data are expressed as mean ± SEM n= 6-7/group

Central Amygdala (CeA)

		Ipsilateral								Contralateral							
		Neurotransmitters (nmol/g)				ECs/NAEs (nmol/g)				Neurotransmitters (nmol/g)				ECs/NAEs (nmol/g)			
		GABA	Glu	Dopa	5-HT	2-AG	AEA	PEA	OEA	GABA	Glu	Dopa	5-HT	2-AG	AEA	PEA	OEA
NFC	Vehicle	6688.255 ± 901.913	15745.88 ± 1360.90	1.630 ± 0.431	0.414 ± 0.058	4.720 ± 1.934	0.058 ±0.01	0.236 ± 0.083	0.376±0.113	4777.97 ± 754.51	15436.00 ± 166.91	1.587 ± 0.393	1.306 ± 0.326	6.194 ± 1.476	0.089 ± 0.029	0.557 ± 0.209	0.388 ± 0.089
	PPARα	4893.782 ± 714.892	16395.34 ± 2661.67	2.412 ± 0.527	0.368 ± 0.086	4.560 ± 0.671	**0.09510.006***	0.330 ± 0.089	0.555 ± 0.095	5493.577 ± 928.275	15313.70 ± 2799.744	1.973 ± 0.561	1.741 ± 0.477	6.020 ± 1.506	0.043 ± 0.008	0.308 ± 0.057	0.356 ± 0.063
	PPARβ/δ	6497.674 ± 1171.71	16299.54 ± 3387.37	0.91 ± 0.286	0.467 ± 0.088	5.713 ± 1.083	0.052 ± 0.010	0.308 ± 0.051	0.455 ± 0.104	3920.895 ± 625.935	12577.60 ± 1460.53	1.121 ± 0.271	0.775 ± 0.215	**1.889 ± 0.581***	**0.027 ± 0.009#**	**0.168 ± 0.026#**	**0.201 ± 0.028#**
	PPARγ	1663.445 ± 96.283	5696.22 ± 520.05	71.08 ± 10.46	0.842 ± 0.035	8.511 ± 1.009	0.017 ± 0.001	0.572 ± 0.197	0.087 ±0.021	1200.343 ± 100.262	4600.652 ± 375.53	69.355 ± 9.012	0.644 ± 0.034	3.849 ± 0.630	0.039 ± 0.020	0.181 ± ±0.120	0.076 ± 0.019
FC	Vehicle	5020.846 ± 1841.40	**8620.90 ± 1749.90#**	3.064 ± 0.82	**0.495 ± 0.19#**	5.484 ± 1.101	**0.092 ± 0.025**	0.239 ± 0.063	0.438 ± 0.091	**7589.19 ± 933.11**	14110.71 ± 2458.693	2.486 ± 0.636	**1.809 ± 0.549**	5.795 ± 1.950	0.045 ± 0.014	**0.349 ± ±0.105**	**0.310 ± 0.073**
	PPARα	6040.886 ± 1653.67	13838.03 ± 4003.87	**0.432 ± 0.15***	0.572 ± 0.146	5.485 ± 2.083	0.059 ± 0.023	**0.479 ± 0.10***	0.5 ± 0.113	5436.131 ± 1328.544	16852.78 ± 5190.165	**0.827 ± 0.291***	1.080 ± 0.574	4.216 ± 1.535	0.046 ± 0.015	0.226 ± 0.058	0.286 ± 0.079
	PPARβ/δ	5378.569 ± ±619.10	**13123.97 ± 871.15#***	**1.38 ± 0.49**	0.366 ± 0.036	5.554 ± 1.995	**0.034 ± 0.006***	**0.494 ± 0.0075***	0.473 ± 0.099	**3639.58 ± 1582.01***	15371.86 ± 2599.55	**1.393 ± 0.423***	1.469 ± 0.441	5.214 ± 1.126	0.036 ± 0.005	0.332 ± 0.119	0.319 ± 0.071
	PPARγ	1352.471 ± 138.24	3469.409 ± ±469.856	**56.153 ± 9.92**	0.823 ± 0.098	7.698 ± 0.810	0.017 ± 0.002	0.601 ±0.330	0.112 ± 0.017	1234.000 ± 81.594	4758.15 ± 510.51	90.899 ± 14.153	0.761 ± 0.098	3.498 ± 0.482	0.017 ± 0.001	0.126 ±0.079	0.055 ± 0.013



Data are expressed as mean ± SEM n= 6-7/group

Summary & Conclusions

- The systemic and intra-BLA administration of PPARα, PPARβ/δ and PPARγ antagonists did not have any effect on formalin-evoked nociceptive behaviour or fear-conditioned analgesia.

- The systemic administration of PPARα, PPARβ/δ or PPARγ antagonists potentiated conditioned fear-related behaviour in the presence of nociceptive tone. These results were associated with increased levels of GABA in the contralateral BLA and decreased levels of dopamine and increased levels of PEA in the ipsilateral CeA.

- Pharmacological blockade of PPARα or PPARγ, but not PPARβ/δ, in the BLA, potentiated conditioned fear-related behaviour in the presence of nociceptive tone.

These data suggest that PPARα and PPARγ, but not PPARβ/δ, within the BLA, may play a role in the expression and short-term extinction of conditioned fear in the presence of nociceptive tone.