Investigating The Role Of Medial Prefrontal Cortex PPARα Signalling In Formalin-Evoked Nociceptive Responding In Rats

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Introduction: There is evidence to suggest that nociceptive transmission within the CNS is associated with morphological and functional reorganisation of cells in the medial prefrontal cortex (mPFC), which may contribute to the development of chronic pain states [1-2]. The peroxisome proliferator activated receptor (PPAR) α is a member of the nuclear hormone receptor family of ligand-dependent transcription factors and is widely distributed within the CNS [3]. Endogenous ligands of this receptor include the bioactive lipids *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA), both of which are involved in modulating pain processing. Given the emerging role of PPARα in pain processing[4], we hypothesised that alterations in PPARα signalling within the mPFC may underpin changes in formalin-evoked nociceptive behaviour in rats.

The aims of the present study were (1) to complete a comparative molecular and neurochemical analysis of the PPARα signalling system within the medial prefrontal cortex of rats that had received intra-plantar injection of either saline or the noxious chemical formalin and (2) to establish the effects of pharmacological modulation of PPARα in the mPFC of formalin-treated rats.

Methods: Adult male Sprague-Dawley rats (250-300g; n=6 per group) received intra-plantar injection of either saline or formalin (2.5%) into the right hind paw under brief 3% isoflurane anaesthesia. Formalin-evoked nociceptive behaviour was assessed for 30 minutes and rated using EthoVision XT software. The mPFC was harvested post mortem for measurement of levels of the endogenous PPARα ligands, *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA) by liquid chromatography with tandem mass spectrometry and PPARα mRNA by qRT-PCR. In a separate cohort of Sprague-Dawley rats of similar weight (n=7-9 rats per treatment group), stainless steel guide cannulae were stereotaxically implanted bilaterally in the mPFC. 7-8 days post-surgery, the PPARα agonist 2-[[4-[2-[[(Cyclohexylamino)carbonyl](4-yclohexylbutyl) amino]ethyl]phenyl]thio]-2-methylpropanoic acid (GW7647) (0.1,1 and 10µg/0.5µL) or PPARα antagonist N-((2S)-2-(((1Z)-1-Methyl-3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-enyl)amino)-3-(4-(2-(5-methyl-2-phenyl-1,3-oxazol-4- l)ethoxy)phenyl) propyl)propanamide (GW6471) (10µg/0.5µL) or Vehicle (100% DMSO) were administered 10 minutes pre-formalin injection to evaluate the effects of pharmacological modulation of PPARα in the mPFC on formalin-evoked nociceptive behaviour. Formalin-evoked nociceptive behaviour was recorded for 90 minutes and rated using EthoVision XT software. Nociceptive behaviour data were analysed by one-way repeated measures ANOVA with Fisher's LSD post-hoc test. qRT-PCR and LC-MS/MS data were analysed using unpaired student's t-test (Data presented below are means ± SEM; $p<0.05$ considered significant).

Results: Formalin-evoked nociceptive behaviour was associated with a significant reduction in mPFC levels of PEA (saline vs. form: 0.09±0.02 vs. 0.06±0.01 nmol/g tissue weight, $p<0.01$) and OEA (0.12±0.06 vs. 0.067±0.01 nmol/g tissue weight, $p<0.01$). A significant reduction in mPFC PPARα mRNA was also observed in formalin-treated rats (saline vs.

formalin 100±30 vs. 32±1.0, $p<0.01$). Bilateral microinjection of the PPARα agonist GW7647 into the mPFC did not alter formalin-evoked nociceptive behaviour, while the PPARα antagonist GW6471 significantly reduced early second phase formalin-evoked nociceptive behaviour compared with vehicle-treated rats (Composite pain scores:vehicle vs. GW6471 0.7521±0.22 vs. 0.162±0.048, $p<0.05$).

Conclusion: These data suggest that formalin-evoked nociception is associated with a reduction in PPARα signalling in the mPFC and that reduced PPARα tone in the mPFC may attenuate formalin-evoked nociceptive behaviour in rats.

All animal experiments were performed in accordance with the Animal Care and Research Ethics Committee, National University of Ireland, Galway, Irish Department of Health and Children, and the European Communities Council directive 86/609 guidelines on the use of animals in scientific research.

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