Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst043.pdf

The Role Of CB_1 Receptors In The RVM In Mediating The Antinociceptive Effects Of The FAAH Inhibitor URB597 In Wistar-Kyoto Rats

Kieran Rea, Weredeselam Olango, Bright Okine, Kathleen Coyle, Brendan Harhen, Michelle Roche, David Finn. National University of Ireland, Galway, Galway, Ireland

Introduction: The stress-hyperresponsive Wistar-Kyoto (WKY) rat strain exhibits a hyperalgesic phenotype in the formalin test[1] compared with Sprague-Dawley (SD) controls. Our recent research has demonstrated differential formalin-evoked expression of endocannabinoid system genes and tissue levels of endocannabinoids in discrete brain regions of WKY vs. SD rats[2]. The rostral ventromedial medulla (RVM) is a critical anatomical component of the descending inhibitory pain pathway[3,4] and a key neural substrate for endocannabinoid-mediated modulation of pain.

Hypothesis: Enhanced pain-related behavioural responding to intra-plantar formalin injection in WKY rats is mediated by impaired mobilisation of endocannabinoid-CB_1 receptor signalling in the RVM.

Methods: Male Sprague-Dawley (275-350g) and WKY (275-350g) rats were assessed in the formalin test. The involvement of the endocannabinoid system in formalin-evoked nociceptive behaviour was further investigated by intraperitoneal (i.p.) administration of 3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl)-cyclohexylcarbamate (URB597; 0.5mg/kg), an inhibitor of the endocannabinoid-catabolising enzyme, fatty acid amide hydrolase (FAAH), or the CB_1 receptor antagonist/inverse agonist AM251 (1(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide) (3.0mg/kg). In a further study, cannulae were stereotaxically implanted 1mm above the RVM of male WKY rats under 2.5% isoflurane anaesthesia. 5-7 days later, rats received 0.5mg/kg URB597 (i.p.) or vehicle, 45 minutes prior to microinjection of DMSO or AM251 (0.03mM) directly into the RVM. Rats received a 10-minute habituation to the formalin test arena immediately prior to intra-plantar formalin administration (2.5%, 50µl) under brief isoflurane anaesthesia (3%). Rats were then returned to the formalin test arena where their behaviour was recorded and later analysed using Ethovision software. At the end of each study, rats were killed by decapitation, brains were harvested and snap-frozen on dry ice for subsequent measurement of RVM endocannabinoid levels by mass spectrometry, mRNA analysis by RT-qPCR or subsequent verification of cannula placement. Behavioural and neurochemical data were analysed using 2- or 3-way analysis of variance followed by Fisher's LSD post-hoc test. (All data presented are means ± SEM with a significant p value <0.05*, <0.01* or <0.001***).

Results: Increased formalin-evoked nociceptive behaviour was observed in WKY rats over the first 35 minutes of the formalin test (CPS: WKY 1.12±0.06 vs. SD 0.85±0.07***) and this behaviour was associated with increased RVM expression of mRNA for the marker of neuronal activity, *zif268* (% of SD-saline controls; SD 68±7 vs. WKY 115±25*) and lower levels of AEA (SD 8.5±0.5 vs WKY 4.8±0.1 pmol/g tissue*) and 2-AG (SD 9.5±1.2 vs WKY 5.1±1.0 nmol/g tissue*) in the RVM, compared with SD controls.

The hyperalgesic response of WKY rats was suppressed by systemic administration of URB597 for the first 35 minutes of the trial (CPS: 1.12±0.06 vs. 0.57±0.03*) and exacerbated by AM251 from 35 to 70 minutes (CPS: 0.66±0.04 vs. 0.82±0.08***). The suppression of formalin-evoked nociceptive behaviour by URB597 was associated with increased levels of AEA in the RVM of WKY rats only (3.3±0.3 vs. 10.1±3.0 pmol/g tissue*). The

antinociceptive effects of URB597 on formalin-evoked nociceptive behaviour in RVM-cannulated WKY rats (CPS: 0.92±0.1 vs. 0.73±0.03*) were blocked by microinjection of AM251 into the RVM (CPS: 0.97±0.07**).

Conclusions: Together, these behavioural, neurochemical and molecular data indicate that impaired endocannabinoid signalling in the RVM underpins hyper-responsivity to noxious stimuli in a genetic background prone to heightened stress/affect.

- Acknowledgements: This work was funded by grants from Science Foundation Ireland (10/IN.1/B2976) and The Irish Research Council for Science, Engineering and Technology

Key Words: Wistar-Kyoto; URB597; Rostral ventromedial medulla (RVM)

1. Burke NN et al, Neuroscience 171, 1300-1313, 2010

2. Okine B et al., Proceedings of the 22nd Annual Symposium on the Cannabinoids, International Cannabinoid Research Society, P3-47. 2012

3. Suplita RL 2nd et al, Neuropharmacology 49, 1201-1209, 2005.

4. Meng ID et al, Nature 395, 381-383, 1998.

5. Martin WJ et al, Neurosci Lett 242, 33-36, 1998.