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Bilateral Microinjection Of The CB_1 Receptor Agonist ACEA Into The PAG Differentially Modulates Formalin-evoked Nociceptive Behaviour In Sprague-Dawley And Stress Hyper-responsive Wistar-Kyoto Rats

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Wistar-Kyoto (WKY) rats are an inbred rat strain that demonstrate physiological and behavioural hyper-responsivity to stress, as well as enhanced nociceptive responding to visceral (*1*), mechanical and inflammatory stimuli (*2*), compared with Sprague-Dawley (SD) rats. This phenotype makes WKY rats a useful genetic model to study hyperalgesia associated with stress/anxiety. Current evidence suggests that the endocannabinoid system plays an important role in stress-induced hyperalgesia (*3-5*). Our recent research has demonstrated differential formalin-evoked expression of CB_1 receptor mRNA and tissue levels of endocannabinoids in the midbrain periaqueductal grey (PAG) of WKY vs. SD rats (6). In addition SD and WKY strains display differential responsivity in the formalin test of tonic persistent pain following systemic administration of endocannabinoid system modulating drugs (7). The aim of the present study was to investigate the effects of CB_1 receptor activation in the lateral/ventrolateral PAG (l/vlPAG) on formalin-evoked nociceptive behaviour in WKY vs. SD rats.

Male SD and WKY rats (250-350g; n= 5-7) were bilaterally implanted with stainless steel guide cannulae aimed at the l/vl PAG under 2.5% isoflurane anaesthesia. 7-8 days later, rats received bilateral intra-PAG microinjection of either vehicle (saline + 0.04% ethanol) or the CB_1 receptor agonist, arachidonyl-2'-chloroethylamide (ACEA; 0.05, 0.5 or 5pmol; 200nL injection volume per side), after which they were habituated to the formalin test arena for 10 minutes., Rats subsequently received intraplantar injection of formalin (2.5%; 50µl) into the right hind paw under brief 3% isoflurane anaesthesia and were returned to the formalin test arena where nocieptive behaviour was recorded. Behaviour was later rated using Ethovision software for 90 minutes and a composite pain score (CPS) was calculated. Data were analysed with repeated measures ANOVA followed by Fisher's LSD post-hoc analysis. $P<0.05$ was considered statistically significant and data are presented below as mean ± SEM. The experimental protocol was carried out following approval from the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under license B100/3613

WKY rats receiving intral/vlPAG vehicle displayed enhanced formalin-evoked nociceptive responding compared with SD counterparts (CPS: 0.61 ± 0.03, n=5, vs. 0.40 ± 0.09, n=7, $p<0.05$). ACEA (0.05pmol) reduced the duration of the second phase formalin response, at discrete time points, in SD rats, with nociceptive behaviour returning to baseline earlier compared with vehicle-treated rats (CPS: 0.14 ± 0.087, n=9, vs. 0.53 ± 0.14, n=7, $p<0.05$). Conversely, ACEA (5pmol) increased the duration, at discrete time points, of the second phase formalin response in WKY rats, compared with vehicle-treated controls (CPS: 0.45 ± 0.15, n=8, vs. 0.074 ± 0.01, n=5, $p<0.05$). No significant effects were observed at other drug doses.

In conclusion, WKY rats exhibited enhanced formalin-evoked nociceptive responding compared with SD counterparts, which supports previous findings. CB1 receptor activation in

the l/vl PAG reduced the duration of the second phase formalin-evoked nociceptive response in SD rats, while extending the second phase response in WKY rats. These data suggest differential formalin test responsivity of WKY and SD rats following CB1 receptor activation in the PAG.

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