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Impaired endocannabinoid signalling in the rostral ventromedial medulla underpins genotype-dependent hyper-responsivity to noxious stimuli

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ABSTRACT

Pain is both a sensory and an emotional experience, and is subject to modulation by a number of factors including genetic background modulating stress/affect. The Wistar–Kyoto (WKY) rat exhibits a stress-hyper-responsive and depressive-like phenotype and increased sensitivity to noxious stimuli, compared with other rat strains. Here, we show that this genotype-dependent hyperalgesia is associated with impaired pain-related mobilisation of endocannabinoids and transcription of their synthesising enzymes in the rostral ventromedial medulla (RVM). Pharmacological blockade of the Cannabinoid$_1$ (CB_1) receptor potentiates the hyperalgesia in WKY rats, whereas inhibition of the endocannabinoid catabolising enzyme, fatty acid amide hydrolase, attenuates the hyperalgesia. The latter effect is mediated by CB_1 receptors in the RVM. 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.

1. Introduction

The ability to perceive pain and respond appropriately is essential for survival. However, excessive or persistent pain constitutes a major healthcare problem for those who experience it, and for society and economies. Pain is both a sensory and an emotional experience and is subject to modulation by a number of factors. A key factor is the contribution of genetic background and its influence on stress responding and affective processing. An increased understanding of how such factors can influence pain is important from a fundamental physiological perspective, and may also aid the identification of new therapeutic targets for the treatment of persistent pain and its exacerbation by, and/or co-morbidity with, stress-related affective disorders.

The influence of genetic background and stress/affect on pain can be examined by comparing behavioural, neurochemical, and molecular responses to noxious stimuli across different rodent strains. The Wistar–Kyoto (WKY) inbred rat strain exhibits a stress-hyperresponsive and depressive-like phenotype [5,29,63,64] and displays increased sensitivity to visceral and somatic noxious stimuli, compared with other rat strains [5,31,33,71,85,90]. As such, the WKY rat represents a useful model with which to study the impact of genetic background and negative affect on pain processing.

The endogenous cannabinoid (endocannabinoid) system plays a key role in the modulation of both pain processing and emotionality [23,27,38,39,48,87,88]. This system comprises at least 2 receptors, the Cannabinoid$_1$ (CB_1) [14,52] and CB_2 [58] receptors, of which the CB_1 receptor is most abundant in the brain. *N*-arachidonoylethanolamide (anandamide: AEA [15]) and 2-arachidonoylglycerol (2-AG [53,83]) are the 2 most extensively studied endogenous ligands for the cannabinoid receptors. AEA and 2-AG are synthesized from phospholipid precursors by *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) [17,45] and diacylglycerol lipase (DAGL) [75], respectively, and are catablolised primarily by fatty acid amide hydrolase (FAAH) [10,62,76] and monoacylglycerol lipase [MAGL] [30], respectively. Both endocannabinoids have similar affinity for both CB_1 [47] and CB_2 [32]

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receptors; however, 2-AG usually has higher efficacy than AEA at both receptors [32,47]. Work from our laboratory and others has demonstrated an important role for these endocannabinoids in stress–pain interactions, regulating both stress-induced analgesia [6–8,28,60,67,69] and stress-induced hyperalgesia [40,77]. To date, however, the contribution of the endocannabinoid system to altered nociceptive responding in genetic backgrounds predisposed to negative affect has not been investigated. Research has shown that the endocannabinoid system of WKY rats is dysfunctional, with altered expression of endocannabinoid catabolising enzymes likely contributing to their depressive phenotype [86]. However, the extent to which alterations in the endocannabinoid system may explain altered nociceptive responding in WKY rats is unknown, and was the focus of the current studies.

We have used behavioural, neurochemical, and molecular approaches to test the hypothesis that enhanced pain-related behavioural responding to the noxious inflammatory stimulus of intra-plantar formalin injection in WKY rats is mediated by impaired mobilisation of endocannabinoid-CB_1 receptor signalling. Our studies focused on the role of the endocannabinoid system in the rostral ventromedial medulla (RVM), given its key role in top–down descending modulation of pain [21,22,36,91], and evidence that CB_1 receptors in the RVM [37,51] regulate nociceptive processing [50,55,84].

2. Methods

2.1. Animals

For all experiments, male Sprague–Dawley (SD) and/or Wistar–Kyoto (WKY) rats (Harlan, Bicester, UK) were used. Animals were singly housed, and holding rooms were maintained at a constant temperature (21 ± 2°C) under standard lighting conditions (12:12-hour light–dark, lights on from 0800 to 2000 h). Experiments were carried out during the light phase between 0800 and 1700 h. Food and water were available ad libitum. The experiments adhered to the guidelines of the Committee for Research and Ethical Issues of IASP [www.iasp-pain.org/AM/Template.cfm?Section=Animal_Research]. The experimental protocol was carried out after approval by the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under license from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609.

2.2. Experimental design

Three separate experiments were performed. In all experiments, animals were randomly assigned to treatment groups, and the sequence of treatments and testing was also randomised to control for the order of testing. Experiment 1 investigated whether enhanced formalin-evoked nociceptive behaviour in WKY rats vs their SD counterparts was associated with alterations in endocannabinoid levels in the RVM or genes coding for the enzymes and receptors of the endocannabinoid system. A total of 24 male Sprague-Dawley rats and 24 male WKY rats (285–320 g) received an intra-plantar injection of 50 μL formalin (2.5% in 0.9% saline, s.c.) or 0.9% saline (control group) into the right hindpaw immediately after a 10-minute habituation exposure to the formalin test arena. This design resulted in 4 experimental groups, as follows: SD-Saline (SD-Sal); SD-Formalin (SD-Form); WKY-Saline (WKY-Sal); and WKY-Formalin (WKY-Form) (n = 10–12 per group). At the peak of the second phase of the formalin test (30 minutes after formalin injection), rats were killed by decapitation. Brains were removed rapidly and were snap-frozen on dry ice and stored at −80°C before microdissection of the RVM and subsequent analysis of AEA and 2-AG levels using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Microdissected RVM tissue was also analysed by quantitative reverse transcription–polymerase chain reaction (RT-PCR) for expression of genes coding for the CB_1 receptor and for the endocannabinoid-related enzymes NAPE-PLD, DAGLα, FAAH, and MAGL. A separate cohort of rats (n = 6 per group) were treated exactly as described above to generate RVM tissue for western blot analysis of CB_1 receptor expression.

In experiment 2, we investigated the effects of pharmacological blockade of the CB_1 receptor or inhibition of FAAH on formalin-evoked nociceptive behaviour in WKY and SD rats. A total of 32 male Sprague–Dawley rats and 32 male Wistar–Kyoto rats (250–350 g) were assessed in the formalin test, with subjects receiving intraperitoneal (i.p.) injection of the CB_1 receptor antagonist/inverse agonist AM251 (3 mg/kg), the FAAH inhibitor URB597 (0.5 mg/kg) or vehicle (ethanol:cremaphor:saline vehicle in a ratio of 1:1:18; 3 mL/kg) before intra-plantar formalin injection. Rats were habituated to the formalin test arena for 10 minutes before formalin injection. URB597 and AM251 were administered 60 minutes and 30 minutes before formalin injection, respectively, based on previous studies in our laboratory and others demonstrating their in vivo efficacy at these doses and times of administration [1,7,8,34,35,41,44,49]. To control for the different times of injection of the 2 drugs, half of the vehicle-treated control rats received vehicle at 30 minutes and half at 60 minutes before intra-plantar formalin injection. These 2 vehicle-treated cohorts were subsequently combined as 1 group after statistical analysis confirmed that there were no differences between them for any of the experimental parameters examined. This design resulted in 6 experimental groups (n = 6–10 per group): SD-Vehicle [SD-Veh]; SD-AM251 (3 mg/kg) [SD-AM251]; SD-URB597 (0.5 mg/kg) [SD-URB]; WKY-Vehicle [WKY-Veh]; WKY-AM251 (3 mg/kg) [WKY-AM251]; and WKY-URB597 (0.5 mg/kg) [WKY-URB]. At the end of the formalin test (ie, 70 minutes after formalin injection), the rats were killed by decapitation.

Experiment 3 was conducted to investigate whether URB597-mediated suppression of formalin-induced hyperalgesia (result from experiment 2) in the WKY rats is mediated by AEA-induced activation of CB_1 receptors in the RVM. Male WKY rats (280–350 g; Harlan, Bicester, UK) were implanted with stainless steel guide cannulae targeting the RVM. On the test day, URB597 (0.5 mg/kg) or vehicle was administered by i.p. injection 60 minutes before formalin injection. Fifteen minutes before formalin injection, 0.3 μL of AM251 (1 μg/0.3 μL) or dimethylsulfoxide vehicle (DMSO, 100%) was microinjected over 1 minute through an injection needle that protruded 1 mm beyond the tip of the pre-implanted guide cannula, with the aid of a Hamilton microsyringe attached to polyethylene tubing and a Harvard PHD2000 infusion pump (Harvard Apparatus, Kent, UK) as described previously [28,60,67,69,72,73]. The injection needle was left in place for 1 minute more after infusion to allow for drug diffusion away from the injector tip. This dose of AM251 was chosen on the basis of previous studies demonstrating that microinjection of AM251 into different regions of the brain modulated behavioural responses to analgesic compounds in various animal models of pain [18,12,20]. After microinjection of AM251 or DMSO vehicle directly into the RVM, animals were immediately placed in a Perspex arena to habituate for 10 minutes. Animals were subsequently injected with formalin under brief anaesthesia and returned to the formalin test arena for behavioural analysis. They were killed by decapitation at 70 minutes after formalin administration. A 0.3-μL quantity of 1% fast green dye was microinjected via the guide cannula, and brains were rapidly removed, snap-frozen on dry ice, and stored at −80°C before injection site verification. Microdissection of the RVM was performed in conjunction with injection site verification, and the microdissected tissue was analysed for endocannabinoid levels using LC-MS/MS.