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Research report

FAAH inhibition attenuates TLR3-mediated hyperthermia, nociceptive- and anxiety-like behaviour in female rats

Lisa E. Flannery[a,c], Daniel M. Kerr[a,b,c], David P. Finn[b,c], Michelle Roche[a,c,*]

[a] Physiology, School of Medicine, National University of Ireland Galway, Ireland
[b] Pharmacology and Therapeutics, School of Medicine, National University of Ireland Galway, Ireland
[c] NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland Galway, Ireland

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ABSTRACT

Aberrant activation of toll-like receptor (TLR)s results in persistent and prolonged neuroinflammation and has been implicated in the pathogenesis and exacerbation of psychiatric and neurodegenerative disorders. TLR3 coordinates the innate immune response to viral infection and recent data have demonstrated that inhibiting fatty acid amide hydrolase (FAAH), the enzyme that primarily metabolizes anandamide, modulates TLR3-mediated neuroinflammation. However, the physiological and behavioural consequences of such modulation are unknown. The present study examined the effect of URB597, a selective FAAH inhibitor, on neuroinflammation, physiological and behavioural alterations following administration of the TLR3 agonist and viral mimetic poly I:C to female rats. URB597 attenuated TLR3-mediated fever, mechanical and cold allodynia, and anxiety-like behaviour in the elevated plus maze and open field arena. There was no effect of URB597 on TLR3-mediated decreases in body weight and no effect in the sucrose preference or forced swim tests. URB597 attenuated the TLR3-mediated increase in the expression of CD11b and CD68, markers of microglia/macrophage activation. In summary, these data demonstrate that enhancing FAAH substrate levels suppresses TLR3-mediated microglia/macrophage activation and associated changes in fever, nociceptive responding and anxiety-related behaviour. These data provide further support for FAAH as a novel therapeutic target for neuroinflammatory disorders.

1. Introduction

The endocannabinoid system has been shown to exhibit potent immunomodulatory effects and represents a potential therapeutic target for peripheral and central inflammatory disorders [1–5]. *N*-arachidonoylethanolamine (anandamide; AEA), the most studied endocannabinoid to date, mediates its effects via cannabinoid (CB_1 and CB_2) and non-cannabinoid (TRPV1, PPARs and GPR55) receptors and is primarily broken down by fatty acid amide hydrolase (FAAH) [6]. *in vitro* and *in vivo* evidence have demonstrated that FAAH inhibition, and associated increases in AEA and the related *N*-acylethanolamines *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), result in the modulation of inflammatory responses induced following the activation of the pattern recognition receptors, toll-like receptor (TLR)s [for review see [7]. (TLR)3 activation results in the induction of type 1 interferon (IFN-α and IFN-β) and NFκB-inducible (e.g. IL-1β, IL-6 and TNF-α) inflammatory cascades which are responsible for coordinating the innate immune response to viral infection. Recent data has highlighted that FAAH inhibition attenuates the TLR3-mediated increase in the expression of IFN-inducible genes and pro-inflammatory cytokines in brain regions such as the hippocampus and hypothalamus, without altering peripheral immune responses [8,9]. The behavioural and physiological consequences of TLR3 activation include the induction of sickness behaviours such as fever/hypothermia, hypoactivity and anorexia [8,10–13] and enhanced pain sensitivity [14] which represents a highly adaptive coping mechanism by the CNS to fight viral infection. However, aberrant activation of TLR3 can elicit adverse effects on the CNS including increased neuronal excitability and seizure susceptibility [15,16], impaired contextual and working memory [16], anxiety- and depressive-like behaviour [17] and exacerbation of underlying

Abbreviations: AEA, anandamide; ADT, acetone drop test; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CD11b, cluster of differentiation molecule; 11b, CD68, cluster of differentiation 68; CNS, central nervous system; COX-2, cyclooxygenase 2; EPM, elevated plus maze; FAAH, fatty acid amide hydrolase; FST, Forced swim test; GFAP, glial fibrillary acidic protein; IFN, interferon; IL, interleukin; i.p, intraperitoneal; IP-10, interferon gamma-induced protein 10; IRF, interferon regulatory factor; LMA, locomotor activity; LPS, lipopolysaccharide; MRC2, mannose receptor C type 2; NFκB, nuclear factor kappa B; OEA, *N*-oleoylethanolamide; OFT, open field test; PEA, *N*-palmitoylethanolamide; PGE_2, prostaglandin E2; Poly I:CPolyinosinic, polycytidylic acid; SPT, sucrose preference test; TLR, toll-like receptor; TNF, tumour necrosis factor; URB597, [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate; VFT, Von Frey test

* Corresponding author at: Physiology, School of Medicine, National University of Ireland Galway, University Road, Galway, Ireland.
E-mail address: Michelle.roche@nuigalway.ie (M. Roche).

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neurodegenerative processes [18,19]. However, it is unknown if FAAH-induced modulation of TLR3-mediated inflammatory responses result in associated physiological and behavioural changes.

Several studies have demonstrated that FAAH inhibition alters anxiety- [20–25] and depressive-like behaviour [21,26] and elicits analgesic effects [22]. However, few studies have evaluated if similar effects occur in the presence of heightened inflammatory tone. The FAAH substrates AEA, OEA and PEA have been shown to modulate TLR4-induced thermoregulatory changes and hypophagia [27–29], most likely mediated via modulation of hypothalamic cytokine expression [28]. A recent study from our group demonstrated that FAAH inhibition modulated TLR4-mediated neuroinflammatory responses in the hippocampus and frontal cortex, an effect which was accompanied by an attenuation of TLR4-mediated anhedonia, but not sickness behaviour [30]. Furthermore, FAAH inhibition has been demonstrated to reverse TLR4-mediated mechanical allodynia [31], thermal hyperalgesia and paw oedema [32]. Collectively, these results demonstrate a role for FAAH substrates in the modulation of behavioural responses following TLR4 activation, although there are no studies to date examining if similar responses occur following activation of other TLRs such as TLR3. Thus, the aim of the present study was to examine the effect of enhancing FAAH substrate levels on TLR3-mediated neuroimmune activation and resulting physiological and behavioural responses.

2. Experimental procedures

2.1. Animals

Experiments were carried out on female Sprague-Dawley rats (weight, 180–250 g; NUI Galway breeding facility), housed singly for at least 3 days prior to the experiment in transparent plastic bottomed cages (48 cm × 20 cm × 27 cm) containing wood shavings as bedding. The animals were maintained at a constant temperature (21 ± 2 °C) under standard light-dark cycle conditions (12: 12 h light-dark, lights on from 0700 to 1900 h). All experiments were carried out during the light phase between 0800 h and 1800 h. Food and water were available *ad libitum*. Animals were habituated to handling and received an intraperitoneal (i.p.) injection of sterile saline (0.89% NaCl) for 2–3 days before experimentation in order to minimise the influence of the injection procedure on behaviour and biological endpoints and the minimum number of animals used. The experimental protocol was carried out in accordance with the guidelines of the Animal Care and Research Ethics Committee, National University of Ireland Galway under licence from the Irish Department of Health and Children in compliance with the European Communities Council directive 2010/63/EU and ARRIVE guidelines.

2.2. Experimental design

2.2.1. Experiment 1: the effect of FAAH inhibition on poly I:C-induced sickness behaviour, nociceptive responding, anxiety- and depressive-like behaviour

Rats were randomly assigned to one of three treatment groups: Vehicle-saline (n = 6–8), Vehicle-poly I:C (n = 9) and URB597-poly I:C (n = 9). The TLR3 agonist poly I:C (3 mg/kg i.p., GE Healthcare, Ireland) or saline vehicle (0.89% NaCl, i.p.) were administered in an injection volume of 1.5 ml/kg 30 min following systemic i.p. administration of the FAAH inhibitor URB597 (1 mg/kg, Cayman Chemicals, Estonia) or vehicle (ethanol: cremophor: saline; 1:1:18) in an injection volume of 2 ml/kg. The dose and timing of URB597 and poly I:C administration were chosen based on previous published work [8,9,33]. Behavioural responding was assessed over a 24 h period. Sickness behaviour was assessed by recording rectal temperature, home cage locomotor activity and body weight. Nociceptive responding to mechanical and cold stimuli was assessed using the von Frey and acetone drop tests, respectively. Anxiety-like behaviour was assessed in the open field and elevated plus maze and depressive-like behaviour was assessed using the sucrose preference test (anhedonia) and forced swim test (behavioural despair/stress coping behaviour). Separate cohorts of animals were used for behavioural testing. Cohort 1: Temperature, Homecage activity (HCA), Body weight and Open Field Test (OFT). Cohort 2: Sucrose Preference (SPT). Cohort 3: Von Frey test (VFT) and Acetone drop test (ADT), Elevated plus maze (EPM) and Forced Swim test (FST). At the end of behaviour testing, animals from cohort 3 were sacrificed, brain removed and hypothalamus excised and frozen at −80 °C until expression of markers for microglia/macrophage and astrocyte activation.

2.2.2. Experiment 2: the effect of FAAH inhibition on temperature, nociceptive responding and anxiety-like behaviour in the absence of TLR3 stimulation

Rats were randomly assigned to one of two treatment groups: Vehicle-saline (n = 6) and URB597-saline (n = 7). The FAAH inhibitor URB597 (1 mg/kg) or vehicle (ethanol: cremophor: saline; 1:1:18) were administered i.p. in an injection volume of 2 ml/kg followed 30 min later by an i.p, injection of sterile saline (0.89% NaCl) administered in an injection volume of 1.5 ml/kg. Animals were analysed for temperature, nociceptive responding and anxiety behaviour in the EPM.

2.3. Temperature recording and body weight

Body temperature was measured using a rectal probe (Omron EcoTemp Smart Digital Thermometer) prior to any experimental manipulation and 4, 8 and 24 hs post poly I:C/saline injection. Body weight was recorded prior to any experimental manipulation and 24 hs following poly I:C/saline injection and used to calculate body weight gain over the 24 h period.

2.4. Behavioural testing

2.4.1. Homecage locomotor activity monitoring

Home cage locomotor activity was assessed using the Opto-M3 Dual Axis system (Columbus Instruments, Columbus, OH) as previously described [34,35]. Following poly I:C/saline injection, animals were returned to their home cage and horizontal activity (total beam breaks) was recorded and presented as activity during the light phase (0–8 h post poly I:C/saline) and the dark phase (nocturnal activity: 14–22 h post poly I:C/saline).

2.4.2. Nociceptive responding to mechanical and cold stimuli

The arena used for the von Frey test (VFT) and acetone drop test (ADT) consisted of a six-compartment Perspex arena (11 cm × 20 cm × 15 cm) with wire mesh flooring as previously described [36–38]. A modified von Frey behavioural testing was performed to assess mechanical allodynia as previously described [14]. In brief, rats were habituated to the arena for at least 15 min after which time an 8 g von Frey filament (Touch-Test® Sensory Evaluators, North Coast Medical, Inc., Gilroy, CA, USA) was applied perpendicular to the mid-plantar surface of the hindpaw, for up to a maximum of 5 s or until flinching, licking or withdrawal of the paw occurred.

Testing occurred on both right and left hindpaws five times (alternating between paws for a total of ten withdrawals). Results were expressed as the percent response frequency of paw withdrawals (number of withdrawals/10 × 100). Immediately following VFT, animals were assessed for cold allodynia in the ADT. In brief, 0.2 ml of acetone (Sigma-Aldrich, Dublin, Ireland) was applied to the plantar surface of the hindpaw and latency to respond within 60 s was recorded. A positive response was considered as a flinch, lick or withdrawal of the hindpaw. If the animal did not respond within 60 s, this value was taken as the latency. The average of the 3 trials was calculated for each hindpaw.