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Full-length Article

Pharmacological inhibition of FAAH modulates TLR-induced neuroinflammation, but not sickness behaviour: An effect partially mediated by central TRPV1

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ABSTRACT

Aberrant activation of toll-like receptors (TLRs), key components of the innate immune system, has been proposed to underlie and exacerbate a range of central nervous system disorders. Increasing evidence supports a role for the endocannabinoid system in modulating inflammatory responses including those mediated by TLRs, and thus this system may provide an important treatment target for neuroinflammatory disorders. However, the effect of modulating endocannabinoid tone on TLR-induced neuroinflammation *in vivo* and associated behavioural changes is largely unknown. The present study examined the effect of inhibiting fatty acid amide hydrolyase (FAAH), the primary enzyme responsible for the metabolism of anandamide (AEA), *in vivo* on TLR4-induced neuroimmune and behavioural responses, and evaluated sites and mechanisms of action. Systemic administration of the FAAH inhibitor PF3845 increased levels of AEA, and related FAAH substrates N-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), in the frontal cortex and hippocampus of rats, an effect associated with an attenuation in the expression of pro- and anti-inflammatory cytokines and mediators measured 2hrs following systemic administration of the TLR4 agonist, lipopolysaccharide (LPS). These effects were mimicked by central i.c.v. administration of PF3845, but not systemic administration of the peripherally-restricted FAAH inhibitor URB937. Central antagonism of TRPV1 significantly attenuated the PF3845-induced decrease in *IL-6* expression, effects not observed following antagonism of CB_1, CB_2, PPARα, PPARγ or GPR55. LPS-induced a robust sickness-like behavioural response and increased the expression of markers of glial activity and pro-inflammatory cytokines over 24hrs. Systemic administration of PF3845 modulated the TLR4-induced expression of neuroimmune mediators and anhedonia without altering acute sickness behaviour. Overall, these findings support an important role for FAAH substrates directly within the brain in the regulation of TLR4-associated neuroinflammation and highlight a role for TRPV1 in partially mediating these effects.

1. Introduction

Toll-like receptors (TLRs) are key players in host defense, homeostasis and response to injury. However, uncontrolled and aberrant TLR activation has been proposed to trigger the onset of certain psychiatric and neurodegenerative disorders and elicit detrimental effects on the progression and outcome of established disease [for reviews see (Arroyo et al., 2011; Bergink et al., 2014; Deleidi and Isacson, 2012; Reus et al., 2015)]. Furthermore, TLR-induced neuroinflammation results in a constellation of behavioural changes which include altered appetite, reduced mood, cognitive changes, anxiety and anhedonia. Accumulating evidence demonstrates

Abbreviations: 2-AG, 2-arachidonoyl glycerol; CB, cannabinoid; FAAH, fatty acid amide hydrolyase; i.c.v., intracerebroventricular; IL, interleukin; NFκB, nuclear factor kappa B; LPS, lipopolysaccharide; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; TLR, toll-like receptor; TNF, tumour necrosis factor; SOCS, suppressor of cytokine signalling;

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potent immunoregulatory effects of the endogenous cannabinoid (endocannabinoid) system, suggesting that this system may represent an important therapeutic target in disorders with a neuroinflammatory component [for reviews see (Downer, 2011; Fitzgibbon et al., 2015; Henry et al., 2016)]. The most widely studied endocannabinoid, *N*-arachidonoyl ethanolamine (AEA, also referred to as anandamide), has been shown to modulate neuroimmune responses, including those induced following TLR activation, although the effects depend on conditions under investigation. For example, several *in vitro* studies have demonstrated that increasing AEA tone, directly or via inhibition of the primary enzyme responsible for its metabolism, the serine hydrolase fatty acid amide hydrolase (FAAH), is associated with attenuation of TLR4-induced production of pro-inflammatory cytokines and mediators such as TNFα, IL-1β, prostaglandins and nitric oxide (Facchinetti et al., 2003; Molina-Holgado et al., 1997; Ortega-Gutierrez et al., 2005; Puffenbarger et al., 2000; Tham et al., 2007), while concurrently increasing anti-inflammatory mediators such as IL-10 (Correa et al., 2010; Krishnan and Chatterjee, 2012). However, data also demonstrate an augmentation of TLR4-induced pro-inflammatory mediators such as IL-6 by AEA (Molina-Holgado et al., 1998; Ortega-Gutierrez et al., 2005). While some studies have demonstrated anti-inflammatory effects of AEA on TLR4-induced inflammatory responses to be mediated by cannabinoid CB_1 and/or CB_2 receptor activation and consequential regulation of NFκB and MAPK activation (Correa et al., 2009a, 2010; Krishnan and Chatterjee, 2012; Ortega-Gutierrez et al., 2005), non-CB_1/CB_2 receptor mediated effects of AEA on inflammatory processes *in vitro* have also been reported (Correa et al., 2008; Tham et al., 2007). AEA also has affinity for and activity at additional receptor targets to CB_1 and CB_2 receptors, namely the peroxisome proliferator-activated receptors (PPARs), the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) and also the novel cannabinoid receptor, G-protein coupled receptor (GPR) 55 [for reviews see (Alexander and Kendall, 2007; Di Marzo et al., 2001; Madasu et al., 2015; O'Sullivan and Kendall, 2010)], activity at which may account for the variability in the effects of AEA on neuroinflammatory responses following TLR activation.

Similar to *in vitro* data, *in vivo* studies have also revealed modulation of TLR4-induced inflammatory responses by AEA. The proposed AEA reuptake inhibitor AM404 has been shown to attenuate TLR4-induced increases in plasma levels of IL-6 and IL-1β, the latter effect mediated by CB_1 receptor activation (Roche et al., 2008). Furthermore, AM404 or enhancing AEA tone via pharmacological inhibition of FAAH, augmented TLR4-induced increases in plasma TNFα levels, an effect at least partially mediated via activation of PPARγ (Roche et al., 2008). In the brain, AEA activation of hypothalamic CB_1 receptors has been shown to facilitate (De Laurentiis et al., 2010), while antagonism of the central CB_1 receptors attenuates (Steiner et al., 2011), TLR4-induced increases in plasma TNFα levels. In addition, work from our laboratory has demonstrated that enhancing AEA levels following FAAH inhibition was associated with attenuation of TLR4-induced increases in IL-1β, and increases in expression of suppressor of cytokine signalling (SOCS3), in the hypothalamus (Kerr et al., 2012). It should be noted that in addition to AEA, related fatty acid amides, *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), are also metabolised by (FAAH) and shown to be increased following FAAH inhibition. These *N*-acylethanolamines have been shown to exert potent biological effects on satiety, pain and inflammation (Esposito and Cuzzocrea, 2013; Mattace Raso et al., 2014; Sayd et al., 2015; Skaper et al., 2015; Suardiaz et al., 2007; Thabuis et al., 2008) and so it cannot be ruled out that some of the effects of FAAH inhibition may be due in part to activity of OEA or PEA, alone or in combination, with AEA. In addition to AEA, OEA has activity at the TRPV1 (Ahern, 2003; Almasi et al., 2008; Gonzalez-Aparicio and Moratalla, 2014; Movahed et al., 2005; Starowicz et al., 2013; Wang et al., 2005) and increasing evidence supports an important physiological role for TRPV1 in the brain (Edwards, 2014; Madasu et al., 2015; Martins et al., 2014). Furthermore, FAAH inhibition can lead to indirect activation/desensitization of TRPV1 and subsequent analgesic effects, anti-inflammatory effects and central effects on mood (Maione et al., 2007; Rubino et al., 2008; Starowicz et al., 2013). Taken together, data indicate that enhancing AEA (and related fatty acid amides) *in vivo* can modulate neuroinflammatory and behavioural responses. However, it remains to be determined if immunomodulatory effects occur due to indirect modulation of peripheral TLR4-induced immune responses, or directly at the level of the brain. Recent work from our group has demonstrated an important role for central FAAH substrates in attenuating the neuroinflammatory response to TLR3 activation (Henry et al., 2014). However, it is unknown if a similar effect is observed following activation of TLR4, or the receptor and molecular mechanisms involved. Furthermore, it remains to be determined if modulation of TLR4-induced neuroinflammatory responses results in concomitant alterations in behaviour. As such the aim of the current study was to investigate if enhancing FAAH substrate, tone in the brain directly modulates TLR4-induced neuroinflammatory responses, sickness behaviour and anhedonia, and examine the potential receptor and molecular mechanism(s) mediating these effects.

2. Materials and methods

2.1. Animals

Experiments were carried out on male Sprague-Dawley rats (weight, 250–300 g; Charles River, UK) housed singly in plastic bottomed cages (45*25*20 cm) containing wood shavings as bedding. The animals were maintained at a constant temperature (21 ± 2 °C) under standard lighting 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 i.p. injection of sterile saline (0.89% NaCl) for 3–4 days before experimentation to minimise the influence of the injection procedure on biological endpoints. 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 the licence from the Irish Department of Health and Children, and in compliance with the European Communities Council directive 2010/63/EU. All sections of the study adhered to the ARRIVE Guidelines for reporting in animal research (Kilkenny et al., 2010).

2.2. Experimental design

2.2.1. Experiment 1: The effect of systemic administration of the FAAH inhibitor PF3845 on TLR4-induced inflammation in the rat brain

Rats were randomly assigned into one of two treatment groups: Vehicle-LPS or PF3845-LPS (n = 8–10 per group) sacrificed 2 post LPS. The potent FAAH inhibitor PF3845 (NIMH drug synthesis programme; 10 mg/kg, i.p.) or vehicle (ethanol:cremaphor:saline; 1:1:18) was administered 30 min prior to systemic administration of LPS (100 μg/kg, i.p.). The dose of PF3845 was chosen on the basis of previous published work which demonstrated that systemic administration of PF3845 increased brain levels of the FAAH substrates AEA, OEA and induced biological effects in several assays (Ahn et al., 2009; Booker et al., 2012; Nasirinezhad et al., 2015; Rock et al., 2015). Published and pilot work in our laboratory has also demonstrated that FAAH inhibitors do not modulate cytokine expression in the brain in the absence of an immune stimulus (Kerr