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N-acylenoethanolamine regulation of TLR3-induced hyperthermia and neuroinflammatory gene expression: A role for PPARα

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ARTICLE INFO

Keywords:
Viral infection
Cannabinoid
PPAR
PEA
OEA
Anandamide
Temperature
Hypothalamus
Neuroinflammation

ABSTRACT

Increasing evidence suggests that SARS-CoV-2, the virus responsible for the COVID-19 pandemic, is associated with increased risk of developing neurological or psychiatric conditions such as depression, anxiety or dementia. Uncontrolled immune responses to viral infection have been proposed to underlie the pathophysiology and exacerbation of a host of neurological and psychiatric conditions. Thus, unsurprisingly, recent evidence indicates this is also the case following SARS-CoV-2 infection (Harapan and Yoo, 2021; Mahalakshmi et al., 2021; Taquet et al., 2021), which is responsible for coronavirus disease 2019 (COVID-19), a pandemic that has overtaken the world during the past year. Viral antigens mediate immune responses by activating pattern recognition receptors such as toll-like receptor (TLR)3, resulting in induction of type 1 interferon (IFN-α and IFN-β) and NFκB-inducible (e.g. IL-1β, IL-6 and TNF-α) inflammatory cascades responsible for host defences, homeostasis and response to injury. However, uncontrolled and aberrant inflammatory gene expression: A role for PPARα.

1. Introduction

Uncontrolled immune responses to viral infection have been proposed to underlie the pathophysiology and exacerbation of a host of neurological and psychiatric conditions. Thus, unsurprisingly, recent evidence indicates this is also the case following SARS-CoV-2 infection (Harapan and Yoo, 2021; Mahalakshmi et al., 2021; Taquet et al., 2021), which is responsible for coronavirus disease 2019 (COVID-19), a pandemic that has overtaken the world during the past year. Viral antigens mediate immune responses by activating pattern recognition receptors such as toll-like receptor (TLR)3, resulting in induction of type 1 interferon (IFN-α and IFN-β) and NFκB-inducible (e.g. IL-1β, IL-6 and TNF-α) inflammatory cascades responsible for host defences, homeostasis and response to injury. However, uncontrolled and aberrant
activation of TLR3 has been shown to impair contextual and working memory (Baghel et al., 2018; Galic et al., 2009), elicit anxiety- and depressive-like behaviour (Gibney et al., 2013), increase neuronal excitability and seizure susceptibility (Costello and Lynch, 2013; Galic et al., 2009) and exacerbatate underlying neurodegenerative processes (Deleidi et al., 2010; Field et al., 2010). Furthermore, TLR3 expression has been demonstrated to be increased in the brain of patients with neurodegenerative (Walker et al., 2018) and psychiatric (Pandey et al., 2014) disorders. Thus, modulating the neuroinflammatory, and consequently neurological, effects of TLR3 activation is of critical physiological and therapeutic importance.

The cannabinoid system exhibits well recognised immune-modulatory properties (Henry et al., 2016; Russo et al., 2018; Tahamtan et al., 2016). Accordingly, cannabinoids and related N-acylthanolamines such as N-palmitoylethanolamide (PEA) have been proposed as potential therapeutics limiting mast cell activation and inflammatory response to SARS-CoV-2 (Gigante et al., 2020; Lucaciu et al., 2021). Recent data have shown that the plant-derived cannabinoid cannabinoid inhibits SARS-CoV-2 replication and viral gene expression, induces interferon (IFN) expression and up-regulates its antiviral signalling pathways (Nguyen et al., 2021). Similarly, the synthetic cannabinoid agonist WIN55,212 has been shown to increase TLR3-induced IFN-β levels while attenuating pro-inflammatory NFkB-related immune responses in astrocytes (Downer et al., 2011). Increasing endogenous cannabinoid tone by inhibiting the catalolisom enzymes for anandamide and 2-AG, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) respectively, has shown that FAAH, but not MAGL, inhibition attenuates TLR3-induced neuroinflammation, but not peripheral, immune responses (Flannery et al., 2018b; Henry et al., 2014). Furthermore, inhibition of TLR3-induced neuroinflammation following FAAH inhibition is associated with an attenuation of TLR3-associated hyperthermia, anxiety-like behaviour and enhanced nociceptive responding (Flannery et al., 2018b), indicating that FAAH substrates are important modulators of TLR3-induced neuroinflammation and associated behavioural responding. In addition to the endocannabinoid anandamide, FAAH also metabolises the related fatty acid amides, N-oleylethanolamide (OEA) and PEA (Cravatt et al., 1996), and thus, inhibition of FAAH results in increases in all three substrates (Fegley et al., 2005; Flannery et al., 2018a). It is unknown if one or all of these substrates is responsible for modulating the TLR3-induced neuroinflammatory and associated behavioural responding following FAAH inhibition.

The effects of AEA, OEA and PEA on TLR4-induced inflammatory responses have been well documented. AEA attenuates TLR4-induced production of pro-inflammatory cytokines and mediators such as TNF-α, IL-1β, prostaglandins (PG) and nitric oxide (NO) (Facchini et al., 2003; Molina-Holgado et al., 1997; Puffenbarger et al., 2000), while concurrently increasing anti-inflammatory mediators such as IL-10 (Correa et al., 2010; Krishnan and Chatterjee, 2012). N-acylthanolamine acid amidase (NAAA) is a further metabolic pathway for OEA and PEA, inhibition of which elicits potent immunosuppressive effects (Alhouayek et al., 2015; Pionelli et al., 2020; Skaper et al., 2015; Solorzano et al., 2009). PEA reduces TLR4-induced increases in TNF-α production and IL-6 and iNOS expression in macrophages (Li et al., 2012; Solorzano et al., 2009) and inhibits TLR4-induced pro-inflammatory M1 microglia while augmenting anti-inflammatory M2a microglia (D’Aloia et al., 2021). OEA decreases TLR4-induced increases in expression of pro-inflammatory cytokines (TNF-α and COX-2) in macrophages (Fan et al., 2014; Yang et al., 2016). OEA and PEA induce anti-inflammatory effects in a mouse model of colitis, directly via inhibition of TLR4-mediated immune responses (Esposito et al., 2014; Lama et al., 2020). Within the brain, AEA modulates TLR4-induced inflammatory responses, temperature changes and hypophagia (Hollis et al., 2011; Steiner et al., 2011). OEA and PEA attenuated TLR4-induced inflammatory responses, temperature changes and hypophagia (Hollis et al., 2011; Steiner et al., 2011). OEA and PEA attenuated TLR4-induced inflammatory activity, IL-1β, COX-2, mPGES-1 expression and PGE2 levels in the hypothalamus, an effect associated with potentiation of TLR4-induced hypothermia (Sayd et al., 2015). OEA blocks the TLR4-mediated increases in pro-inflammatory cytokines and chemokines, oxidative and nitrosative stress, and neurodegenerative cascades in frontal cortex of a rodent model of alcohol abuse (Orio et al., 2018; Rivera et al., 2019) and neuropsychiatric conditions (Moya et al., 2021). Collectively, this demonstrates that AEA, OEA and PEA modulate TLR4-induced inflammatory responses; however, there is a paucity of studies investigating the effects of individual N-acylthanolamines on TLR3-induced inflammatory responses. PEA has been shown to inhibit TLR3-induced increase in the expression and release of the chemokine MCP-1 in keratinocytes (Petrosino et al., 2010). TLR3 plays a key role in the induction of the TMEV-model of multiple sclerosis, and FAAH inhibition, AEA and PEA has been shown to attenuate microglial activation, the expression of pro-inflammatory cytokines and ameliorates motor symptoms in this model (Mestre et al., 2005; Ortega-Gutiérrez et al., 2005; Loría et al., 2008; Loría et al., 2010; Correa et al., 2011; Herngomez et al., 2012). However, effects of individual FAAH substrates on the acute TLR3-mediated neuroimmune responses and associated sickness behaviour has not been examined. Enhancing FAAH substrate levels inhibits TLR3-induced hyperthermia without altering other aspects of the acute sickness response (Flannery et al., 2018b). As such, this study examined the effects of intracerebral or systemic administration of meth-AEA, OEA and PEA on TLR3-induced hyperthermia and expression of neuroinflammatory genes. OEA and PEA elicit their anti-inflammatory and neuroprotective effects mainly through the activation of nuclear peroxisome proliferator-activated receptor-alpha (PPAR-α) (Di Cesare Mannelli et al., 2013; Gonzalez-Aparicio et al., 2014; Lo Verme et al., 2005; Rankin and Fowler, 2020; Zhou et al., 2012). As such, the role of PPARs on OEA-mediated modulation of TLR3-induced hyperthermia and inflammatory gene expression was also examined.

2. Methods

2.1. Animals

Experiments were carried out on female Sprague-Dawley rats (weight, 200-350 g; In house bred), housed singly in transparent plastic bottomed cages on a constant temperature (21 ± 2°C) under standard light-dark cycle conditions (12: 12 h light-dark, lights on from 0800 to 2000 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 3–4 days before experimentation in order to minimise the influence of the injection procedure on behaviour and 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 licence from the Irish Health Products regulatory Authority and in compliance with the European Communities Council directive 2010/63/EU.

2.2. Experimental design

2.2.1. Experiment 1: the effect of methanandamide on TLR3-induced hyperthermia and neuroinflammatory gene expression

Rats were randomly assigned to one of three treatment groups: Vehicle-Saline (n = 6), Vehicle-Poly I:C (n = 9), Methanandamide (meth-AEA)-Poly I:C (n = 10). Meth-AEA (20g/kg, Abcam, UK) or Vehicle (100% DMSO) were administered in a single acute i.c.v. injection, in an injection volume of 4 μl. This was followed 10 min later by an i.p. injection of poly I:C (3 μg/kg) or sterile saline (0.89% NaCl) administered in an injection volume of 1.5 mg/kg. Due to the rapid metabolism of AEA in vivo, the stable AEA analogue meth-AEA was administered directly to the brain (i.c.v). The concentration of meth-AEA used was chosen based on previous literature demonstrating antinociceptive and gastroprotective effect when administered centrally (Garzon et al., 2009;
2.2.2. Experiment 2: the effects of OEA and PEA on TLR3-induced hyperthermia and neuroinflammatory gene expression

Rats were randomly assigned to one of four treatment groups: Vehicle-saline (n = 8), Vehicle-poly I: C (n = 9), OEA-poly I: C (n = 8) and PEA-poly I: C (n = 9). OEA and PEA (20 mg/kg, Abcam, UK) or Vehicle (ethanol: Cremophor: saline; 1:1:18) were administered i.p. in an injection volume of 2 ml/kg followed 10 min later by an i.p. injection of poly I: C (3 mg/kg) or sterile saline (0.89% NaCl) administered in an injection volume of 1.5 ml/kg. The dose of OEA was chosen as this has been shown to increase striatal levels of OEA from 15 min to 2 h post administration (Gonzalez-Aparicio et al., 2014; Plaza-Zabala et al., 2010) and pilot data in the lab demonstrated increased OEA concentration in the hypothalamus 1 h post administration. The dose of PEA was chosen based on published data demonstrating efficacy in reducing nociceptive behaviour (Pessina et al., 2015). Temperature was recorded prior to injection and 4 h post poly I: C/saline administration. Animals were sacrificed by decapitation at 4 h post-poly I: C/saline administration, the hypothalamus excised, snap-frozen on dry ice and stored at -80 °C until assayed for expression of inflammatory mediators.

2.2.3. Experiment 3: the effects of PPARα antagonism, in the presence and absence of OEA, on TLR3-induced hyperthermia and neuroinflammatory gene expression

Rats were randomly assigned to one of four treatment groups: Vehicle-Vehicle-saline (n = 8), Vehicle-poly I: C (n = 9), Vehicle-OEA-poly I: C (n = 8) and GW6471-Vehicle-poly I: C (n = 7). OEA (20 mg/kg, Abcam, UK) and GW6471 (2 mg/kg) were dissolved in Vehicle (ethanol: cremophor: saline; 1:1:18) were administered i.p. in an injection volume of 2 ml/kg. GW6471 or Vehicle was administered 20 min prior to administration of OEA or vehicle followed 10 min later by an i.p. injection of poly I: C (3 mg/kg) or sterile saline (0.89% NaCl) in an injection volume of 1.5 ml/kg. The dose of GW6471 was chosen based on efficacy in reversing PEA-induced protective effects (Pessina et al., 2015; Scuderi et al., 2014), without affecting nociceptive responding (Gaspar et al., 2020) or anxiety-like behaviour (unpublished in-house data). Temperature was recorded prior to injection and 4 h post poly I: C/saline administration, the spleen and hypothalamus excised, snap-frozen on dry ice and stored at -80 °C until assayed for inflammatory gene expression.

2.2.4. Experiment 4: the effects of PPARα agonism on TLR3-induced hyperthermia and neuroinflammatory gene expression

Rats were randomly assigned to one of three treatment groups: Vehicle-saline (n = 6), Vehicle-poly I: C (n = 8), Vehicle-WY14463 (n = 6). WY14463 (20 mg/kg, Abcam, UK) was dissolved in Vehicle (10% DMSO) and administered i.p. in an injection volume of 2 ml/kg, followed 30 min later by an i.p. injection of poly I: C (3 mg/kg) or sterile saline (0.89% NaCl) in an injection volume of 1.5 ml/kg. The dose of WY14463 was chosen based on in vivo efficacy in several models (Jyme et al., 2019; Okine et al., 2015; Song et al., 2016). Temperature was recorded prior to injection and 4 h post poly I: C/saline administration, after which animals were sacrificed hypothalamus excised, snap-frozen on dry ice and stored at -80 °C until assayed for gene expression.

2.3. Intracerebroventricular (i.c.v.) guide cannula implantation

Intracerebroventricular (i.c.v.) guide cannulae were implanted into the rat brain as previously described (Henry et al., 2014). In brief, under isoflurane anaesthesia (1–3% in O2; 0.5 L/min), a guide cannula (5 mm, Plastics One Inc., Roanoke, Virginia, USA) was stereotaxically implanted into the right lateral ventricle (coordinates: AP: −0.07 mm; ML: −0.15 mm, DV: −0.30 mm; (Paxinos, 2006)). The cannula was permanently fixed to the skull using stainless steel screws and dental acrylic cement and the guide remained patent by the insertion of a stainless steel stilette (Plastics One Inc., USA). Animals received the broad spectrum antibiotic enrofloxacin (2.5 mg/kg s.c.; Baytril, Bayer Ltd., Ireland) on the day of and for 3 days post surgery. Correct cannula placement was verified by the Angiotensin (Ang) II drinking test 3 days prior to the experiment. Animals were considered non-responders if they drank <3mls over 20 min post AngII infusion and were not included in the experiment. Over all experiments, the average number of non-responders was <5%. Animals were allowed to recover from surgery for at least 6 days prior to experimentation.

2.4. Expression of inflammatory mediators using quantitative real-time PCR

RT-qPCR was performed as previously described (Flannery et al., 2018a; Flannery et al., 2018b; Henry et al., 2014). In brief, mRNA was isolated from hypothalamic tissue using NucleoSpin RNA II total RNA isolation kit (Macherey-Nagel, Germany) and reverse transcribed into cDNA using a High Capacity cDNA Archive kit (Applied Biosystems, UK). Taqman gene expression assays (Applied Biosystems, UK) were used to quantify the gene of interest and real-time PCR was performed using an ABI Prism 7500 instrument (Applied Biosystems, UK). Assay IDs were as follows: IP-10 (R00594464_m1), IRF7 (R01450778_g1), TNFα (R00999901_m1), IL-1β (R00580432_m1), IL-10 (R00563409_m1), iNOS (NOS2) (R00561646_m1), COX-2 (R01483828_m1), m-PGE-s (R00572047_m1), SOCS1 (R00595838_s1) and SOCS3 (R00585674_s1). β-actin was used as an endogenous control to normalise gene expression data. Relative gene expression was calculated using the ∆∆CT method.

2.5. Statistical analysis

Data were analysed and graphs using Graph Pad Prizm v9. Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene’s test, respectively. Data were analysed by One-Way ANOVA followed by Student Newman Keules (SNK) post hoc analysis where appropriate. The level of significance was set at p < 0.05. Data are expressed as group means ± standard error of the mean (SEM).

3. Results

3.1. Meth-AEA does not alter TLR3-induced hyperthermia or inflammatory gene expression in the hypothalamus

The data revealed that poly I:C-induced an increase in temperature (F(2,19) = 6.35, p = 0.007) and IP-10 (F(2,19) = 29.97, p < 0.001), TNFα (F(2,19) = 4.89, p = 0.019) and IL-1β (F(2,19) = 3.73, p = 0.044) expression in the hypothalamus, 4 h post administration (Fig. 1a-d). Meth-AEA (i.c.v.) did not alter poly I:C-induced hyperthermia or inflammatory gene expression in the hypothalamus (Fig. 1a-d).

3.2. OEA and PEA attenuates TLR3-induced hyperthermia, but only OEA attenuates TLR3- induced inflammatory gene expression in the hypothalamus

The data revealed that poly I:C significantly increased temperature (F(2,12) = 5.842, p = 0.004). Systemic administration of either OEA or PEA prevented poly I:C-induced hyperthermia (Fig. 2a).

Analysis revealed a significant effect of treatment on the hypothalamic expression of IFN-inducible genes IP-10 (F(3,25) = 24.32, p < 0.001) and IRF7 (F(3,25) = 25.6, p < 0.001), and the NFκB-inducible
Fig. 1. The effect of meth-AEA (i.c.v.) on poly I:C induced (a) hyperthermia and increases in (b) IP-10, (c) TNF-α and (d) IL-1β expression in the hypothalamus, 4 h post poly I:C administration. Data expressed as mean ± SEM (n = 5–9 per group). *p < 0.05; ** p < 0.01 vs vehicle-saline-treated counterparts.

Fig. 2. The effect of OEA or PEA on poly I:C induced (a) hyperthermia and increases in inflammatory gene expression of (b) IP-10, (c) IRF7, (d) TNF-α, (e) IL-1β, (f) IL-10, (g) iNOS, (h) COX2, (i) MPGES, (j) SOCS1 and (k) SOCS3 in the hypothalamus, 4 h post poly I:C administration. Data expressed as mean ± SEM (n = 6–8 per group). ***p < 0.001; ** p < 0.01; * p < 0.05 vs vehicle-saline-treated counterparts. ++p < 0.01; +p < 0.05 vs vehicle-poly I:C-treated counterparts.
genes TNF-α [F(3,25) = 11.03, p < 0.001], IL-1β [F(3,25) = 9.02, p < 0.001], IL-10 [F(3,25) = 8.32, p < 0.01]. Post hoc analysis revealed that poly I:C-induced a significant increase in the expression of all inflammatory genes examined in the hypothalamus compared to vehicle-saline-treated counterparts, 4 h post administration (Fig. 2b-f). OEA significantly attenuated the poly I:C-induced increase in IP-10, IRF7, TNF-α and IL-1β, but not IL-10, expression in the hypothalamus. In contrast, systemic administration of PEA did not alter the poly I:C-induced increase in neuroinflammatory gene expression in the hypothalamus (Fig. 2b-f).

In order to determine if the effects of OEA on poly I:C-induced hyperthermia are accompanied by an attenuation of COX2-PEG2 activity, the expression of genes regulating this pathway was also examined. Analysis revealed a significant effect of treatment on expression of iNOS [F(3,25) = 8.506, p < 0.01], COX2 [F(3,24) = 20.06, p < 0.01] and MPEGS [F(3,25) = 20.47, p < 0.01]. Post hoc analysis revealed that poly I:C induced an increase in expression of iNOS, COX2 and MPEGS, an effect attenuated by OEA, but not PEA (Fig. 2g-i). Furthermore, analysis revealed a significant effect of treatment on the expression of the regulatory genes SOCS1 [F(3,25) = 22.51, p < 0.01] and SOCS3 [F(3,25) = 17.57, p < 0.01] and confirmed that poly I:C-induced an increase in expression of SOCS1 and SOCS3, an effect attenuated by OEA, but not PEA (Fig. 2j-k).

3.3. OEA or PEA do not alter TLR3-induced inflammatory gene expression in the spleen

In order to determine if the effect of OEA on inflammatory gene expression in the hypothalamus are due to modulation of peripheral immune responses following TLR3 activation, inflammatory gene expression was also examined in the spleen. Poly I:C-induced a significant increase in IP-10 [F(3,25) = 129.8, p < 0.001], IRF7 [F(3,25) = 104.1, p < 0.001], TNF-α [F(3,25) = 25.46, p < 0.001] and IL-1β expression [F(3,25) = 16.59, p < 0.001] in the spleen, an effect not altered by OEA or PEA (Fig. 3).

3.4. PPARα antagonism blocks the OEA-induced attenuation of inflammatory gene expression in the hypothalamus following TLR3 activation

Several studies have demonstrated that anti-inflammatory effects of OEA have been attributed to activation of PPARα. Thus, the role of PPARα in mediating the effects of OEA on TLR3-induced hyperthermia and neuroinflammatory gene expression in the hypothalamus were examined in the current study. Analysis revealed that poly I:C-induced an increase in body temperature 4 h post administration (P = 0.05), which was not observed in rats that received OEA and/or the PPARα antagonist GW6471 (Fig. 4a). Poly I:C significantly increased the expression of IP-10 [F(4,33) = 10.86, p < 0.001], IRF7 [F(4,33) = 11.31, p < 0.001], TNF-α [F(4,33) = 5.13, p = 0.002] and IL-1β [F(4,33) = 4.52, p = 0.005] in the hypothalamus, an effect not observed in rats pre-treated with OEA (Fig. 4b-e). Administration of GW6471 blocked the effects of OEA on inflammatory gene expression following poly I:C administration. There was no significant effect of GW6471 alone on poly I:C-induced inflammatory gene expression in the hypothalamus (Fig. 4b-e).

In order to determine if the effects of OEA on TLR3-induced responses could be mimicked by PPARα agonism, the effects of systemic administration of the PPARα agonist WY14643 were examined. WY14643 did not alter TLR3-induced hyperthermia, IP-10 or TNFα expression in the hypothalamus (Fig. 5).

4. Discussion

N-acylethanolamines exhibit potent anti-inflammatory effects, however, effects on viral-mediated immune responses within the brain have not been extensively examined. The present study demonstrated that OEA and PEA, but not AEA, attenuate TLR3-induced hyperthermia and OEA attenuates the expression of IFN-α and NFκB-related genes in the hypothalamus, including hyperthermic related genes (IL-1β, iNOS, COX2 and m-PGES). Antagonism of PPARα prevented the OEA-induced attenuation of IFN-α and NFκB-related genes in the hypothalamus following TLR3 activation, without altering temperature. However, PPARα agonism did not alter TLR3-induced hyperthermia or hypothalamic inflammatory gene expression. While the mechanisms mediating the effects of PEA on TLR3-mediated hyperthermia remain to be determined, the data indicate that OEA attenuates TLR3-induced neuroinflammation and hyperthermia, an effect partially mediated by PPARα.

In line with previous data, (Cunningham et al., 2007; Flannery et al., 2018a; Flannery et al., 2018b; Murray et al., 2015), the present study confirms that poly I:C-induced activation of TLR3 elicits a robust induction of IFN-α and NFκB-mediated immune responses both peripherally and centrally, accompanied by hyperthermia. Increasing FAAH substrate levels has been demonstrated to attenuate TLR3-induced hyperthermic and neuroinflammatory responses, effects specifically mediated at the level of the central nervous system (Flannery et al., 2018a; Flannery et al., 2018b; Henry et al., 2014). The current data demonstrate that OEA and PEA, but not meth-AEA, attenuate TLR3-induced hyperthermia. In comparison, AEA, OEA and PEA have been shown to modulate TLR4-induced hypo- (Sayed et al., 2015; Steiner et al., 2011) or hyper-thermia (Hollis et al., 2011), although no effect was observed when all 3 substrates are enhanced following FAH inhibition (Henry et al., 2017). It is possible that competitive inhibition exists when all three FAAH substrates are enhanced which overrides the effects of individual N-acylthanolamines on TLR4-induced changes in core body temperature. Such competitive inhibition between FAAH substrates may not take place in response to TLR3 activation, as AEA does not play a significant role in TLR3-mediated thermoregulation. Accordingly, AEA-induced activation of CB1 receptors plays a key role in the thermoregulatory response following TLR4 activation (Duncan et al., 2013; Fraga et al., 2009; Steiner et al., 2011). In comparison, TLR3-mediated hyperthermia is maintained in CB1−/− mice (Duncan et al., 2013), a finding.
further supported by unpublished data from our lab demonstrating a lack of effect of central CB1 or CB2 receptor agonism on TLR3-induced hyperthermia. Thus, taken together, these data suggest that AEA-CB1 receptor activation plays a key role in TLR4-, but not TLR3-, induced thermoregulatory and neuroinflammatory responses.

OEA and PEA modulate the TLR4-induced hypothermic response, an effect associated with an attenuation in hypothalamic IL-1β, COX-2, mPGES-1 expression and PGE2 levels (Sayd et al., 2015). Similarly, the TLR3-induced hyperthermic response has been shown to be primarily mediated by the IL-1β-COX2 pathway (Fortier et al., 2004). The current study demonstrated that OEA and PEA attenuates TLR3-induced hyperthermia; however, only OEA attenuates the hypothalamic expression of hyperthermic related genes (IL-1β, COX2, iNOS and m-PGES-1). Published and pilot data have demonstrated that OEA crosses the blood brain barrier and increases OEA levels in the brain 20 mins following i.p. administration (Gonzalez-Aparicio et al., 2014) and can remain elevated up to 2 h post injection (Plaza-Zabala et al., 2010). Furthermore, OEA did not alter the expression of TLR3-induced inflammatory genes in the spleen. Thus, it is likely that OEA acts directly at the level of the hypothalamus to attenuate the TLR3-induced activation of the IL1β-COX2-PGE2 pathway and consequently, the associated hyperthermia. The neuro-immuno-modulatory effects of FAAH inhibition following TLR3 activation have been demonstrated to be mediated directly at the level of the brain (Flannery et al., 2018a; Henry et al., 2014). Thus, given the lack of effect of meth-AEA or PEA on TLR3-induced neuroimmune mediators, it is likely that OEA is the primary FAAH substrate modulating TLR3-induced neuroinflammation and associated hyperthermia. The anti-inflammatory effects of OEA are primarily mediated by PPARα (Russo et al., 2018; Xu et al., 2016) and accordingly, the current study demonstrated that PPARα antagonism blocked the inhibitory effect of OEA on TLR3-induced inflammatory gene expression in the hypothalamus. However, PPARα antagonism failed to alter the inhibitory effect of OEA on TLR3-induced hyperthermia, and PPARα agonism failed to modulate TLR3-induced hyperthermia or hypothalamic gene expression, indicating additional receptor (TRPV1, GPR55) or molecular targets and/or thermoregulatory mechanisms are likely to be also involved in mediating the effects of OEA.

Although PEA attenuated TLR3-induced hyperthermia, no effect was observed on the expression of inflammatory genes in the hypothalamus, suggesting differential mechanisms underlie the effects of OEA and PEA on TLR3-induced hyperthermia. We cannot rule out that PEA may have induced effects on hypothalamic inflammatory gene expression at an earlier timepoint than examined in this study. PEA has been reported to cross the blood brain barrier after an oral administration, although at

Fig. 4. The effect of GW6471 on OEA-induced changes in (a) temperature and (b) IP-10, (c) IRF7, (d) TNF-α and (e) IL-1β expression in the hypothalamus. Data expressed as mean ± SEM (n = 7–8 per group). *p < 0.05; **p < 0.01 vs Veh-Veh-Saline. ***p < 0.001 vs Veh-OEA-poly I:C. $p < 0.05 vs Veh-OEA-poly I:C.

Fig. 5. The effect of systemic administration of WY14643 on poly I:C induced (a) hyperthermia and (b) increases in (b) IP-10 and (c) TNF-α expression in the hypothalamus. Data expressed as mean ± SEM (n = 6–8 per group). ***p < 0.001 **p < 0.01 *p < 0.05 vs vehicle-saline.
very low concentrations (<1%) (Artamonov et al., 2005) and unpublished pilot data from our lab suggest that PEA levels were not elevated in the hypothalamus 1 h following administration. It should be noted that in addition to FAAH, OEA and PEA are also hydrolysed by NAAA, the inhibition of which has been shown to elicit potent immunosuppressive activity (Alhouayek et al., 2015; Piomelli et al., 2020; Skaper et al., 2015; Solorzano et al., 2009). NAAA is highly expressed in cells of the immune system and thus, the lack of effect of PEA on hypothalamic gene expression may be due to low central tissue distribution due to its rapid metabolism by NAAA under inflammatory conditions. The effects of PEA on TLR3-induced hyperthermia is most likely mediated peripherally rather than at the level of the hypothalamus. However, the current study demonstrated that PEA did not alter the TLR3-induced increase in IFN- or NFKb-related gene expression in the spleen, indicating that thermoregulatory effects are not merely due to global inhibition of peripheral immune responses to TLR3 activation. However, PEA may have altered the transcription or translation of these genes, the release of cytokines and cytokine inducers stimulate prostaglandin E2 E2 into the brain. Pfloghues Arch. 442, 526–533.

Deleli, M., Hallett, P.J., Kopich, J.B., Chung, C.Y., Isacson, O. 2010. The toll-like receptor-3 agonist poly(I:C) triggers nigrostriatal dopaminergic degeneration. J. Neurosci. 30, 16091–16101.

to Cesare Mancellari, L., Agostino, G., Pachini, A., Russo, R., Zanardelli, M., Ghelardini, C., Calignano, A., 2006. Palmitoylethanolamide is a disease-modifying agent in peripheral neuropathy: pain relief and neuroprotection share a PPAR-alpha-mediated mechanism. Mediat. Inflamm. 2013, 328799.

Downer, E.J., Clifford, E., Gran, B., Nel, H.J., Fallon, P.G., Moyaugh, P.N., 2011. Identification of the synthetic cannabinoid f(-)-WIN55212-2 as a novel regulator of IFN regulatory factor 3 activation and IFN-beta expression: relevance to therapeutic effects in models of multiple sclerosis. J. Biol. Chem. 286, 10316–10328.

Duncan, M., Galic, M.A., Wang, A., Chambers, A.P., McCafferty, D.M., McKay, D.M., Sharpe, K.A., Pittman, Q.J., 2013. Cannabinoid 1 receptors are critical for the innate immune response to TLR4 stimulation. Am. J. Phys. Regul. Integr. Comp. Phys. 305, R224-R231.

Esposito, G., Capocci, E., Turco, F., Palmoumo, L., Lu, J., Steardo, A., Cuomo, R., Sarrelli, G., Steardo, L. 2014. Cannabinoid 1 receptors are critical for pain relief and proinflammatory mediator profile induced by systemic challenge of rats. Behav. Brain Res. 353, 11.

Fan, A., Wu, X., Wu, H., Li, L., Huang, K., Zhu, Y., Qiu, Y., Fu, J., Ren, J., Zhu, C., 2014. Atheroprotective effect of Oleoylethanolamide (OEA) targeting oxidized LDL. Peptides One 9, 483537.

Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarziga, G., Piomelli, D., 2005. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carboxamic acid 3-carboxamido-biphenyl-3-yi ester (URB927): effects on anandamide and oleoylethanolamide deactivation. J. Pharmacol. Exp. Ther. 313, 352–358.

Field, R., Campion, S., Warren, C., Murray, C., Fallon, P.G., Moynagh, P.N., 2011. Systemic challenge with the TLR3 agonist poly I:C induces amplified IFNalpha/beta and IL-beta expression in the diseased brain and exacerbates chronic neurodegeneration. Brain Behav. Immun. 24, 996–1007.

Flannery, L.E., Henry, R.J., Kerr, D.M., Finn, D.P., Roche, M., 2018a. FAAH, but not MAGL, inhibition modulates acute TLR3-induced neuroimmune signalling in the rat, independent of sex. J. Neurosci. Res. 96, 989–1001.

Flannery, L.E., Kerr, D.M., Finn, D.P., Roche, M., 2018b. FAAH inhibition attenuates TLR3-mediated hyperthermia, nociceptive- and anxiety-like behaviour in female rats. Behav. Brain Res. 353, 11–20.

Fortier, M.E., Kent, S., Ashdown, H., Poole, S., Boksa, P., Luheishi, G.N., 2004. The viral mimic, polyinosinic-polycytidylic acid induces fever in rats via an interleukin-1-dependent mechanism. Am. J. Phys. Regul. Integr. Comp. Phys. 287, R759-R766.

Fraga, D., Zanoni, C.I., Rae, G.A., Parada, C.A., Souza, G.E., 2009. Endogenous cannabinoids induce fever through the activation of CB1 receptors. Br. J. Pharmacol. 157, 1494–1501.

Galic, M.A., Rizai, K., Henderson, A.K., Tsutui, S., Pittman, Q.J., 2009. Viral-like brain inflammation during development causes increased seizure susceptibility in adult rats. Neurobiol. Dis. 36, 343–351.

Garzon, J., de la Torre-Madrid, E., Rodriguez-Munoz, M., Vicente-Sanchez, A., Sanchez-Belazquez, P., 2009. Gz mediates the longlasting desensitization of brain CB1 receptors and is essential for cross-tolerance with morphine. Mol. Pain 5, 11.

Gaspard, J.C., Okine, B.N., Loizzi, M., Mancellari, A., Roche, M., Finn, D.P., 2013. Pharmacological blockade of PPAR isoforms increases conditional fear responding in the presence of nociceptive tone. Molecules 25.

Gibney, S.M., McGuinness, B., Prendergast, C., Harkin, A., Connon, T.J., 2013. Poly I:C-induced activation of the hyperthermic response is accompanied by depression and anxiety-like behaviours, kyureneine pathway activation and reduced BDNF expression. Brain Behav. Immun. 28, 170–181.

Gigadi, A., Aguiló, A., Farinelli, L., Caraffa, A., Boncon, G., Enrica Gallenga, C., Tete, G., Kriša, S.K., Conte, P., 2020. Sodium chloride- and palmitoylethanolamide: a molecular characterization of an enzyme that degrades neuromodulatory fatty-acid ethanols in mouse Nature 384, 83–87.

Cunningham, C., Campion, S., Teeling, J., Felton, L., Perry, V.H., 2007. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav. Immun. 21, 900–902.

D'Aloia, A., Molteni, L., Gullo, F., Bresciani, E., Artusa, V., Rizzi, L., Ceriani, M., Meanti, R., Lecchi, M., Coco, S., Costa, B., Torsello, A., 2021. Palmitoylethanolamide modulation of microglia activation: characterization of mechanisms of action and implication for its Neuroprotective effects. Int. J. Mol. Sci. 22.

Davidson, J., Abul, H.T., Milton, A.S., Rotondo, D., 2001. Cytokines and cytokine inducers stimulate prostaglandin E2 E2 into the brain. Pfloghues Arch. 442, 526–533.
Lysne, V., Bjorndal, B., Grinna, M.L., Midttun, O., Ueland, P.M., Berge, R.K., Dierkes, J., Lucaciu, O., Aghiorghiesei, O., Petrescu, N.B., Mirica, I.C., Benea, H.R.C., Apostu, D., Loria, F., Petrosino, S., Mestre, L., Spagnolo, A., Correa, F., Hernangomez, M., et al., Li, Y., Yang, L., Chen, L., Zhu, C., Huang, R., Zheng, X., Qiu, Y., Fu, J., 2012. Design and synthesis of potent N-acylethanolamine-hydrolyzing acid amidase (NAAA) inhibitor (NAAA) alpha-anti-inflammatory compounds. PLoS One 7, e43023.

Hollis, J.H., Jonaidi, H., Lemus, M., Oldfield, B.J., 2011. The endocannabinoid system. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 36, 167–180.

Henry, R.J., Kerr, D.M., Finn, D.P., Roche, M., 2014. FAAH-mediated modulation of endocannabinoid metabolism in the rat hippocampus. J. Neuroimmunol. 276, 126–134.

Henry, R.J., Kerr, D.M., Finn, D.P., Roche, M. For whom the endocannabinoid blows: the role of the endocannabinoid system in the pathogenesis of inflammatory conditions. In: C. P. (Eds.), The Endocannabinoid System: Basic Research and Clinical Applications. Elsevier, Oxford.

Gonzalez-Aparicio, R., Blanco, E., Serrano, A., Pavon, F.-J., Parsons, L.H., Maldonado, R., 2005. Modulation of N-acylethanolamine-hydrolyzing acid amidase (NAAA): structure, function, and inhibition. J. Med. Chem. 48, 7435–7451.

Robledo, P., 2010. Effects of the endogenous PPAR-α agonist, oleoylethanolamide on MDMA-induced cognitive deficits in mice. Synapse 64, 379–389.

Puffenbarger, R.A., Booth, A.C., Cabral, G.A., 2000. Cannabinoids inhibit LPS-inducible synthesis of cytokine mRNA expression in rat microglial cells. Glia 29, 58–69.

Rankin, L., Fowler, C.J., 2020. The basal pharmacology of palmitoylethanolamide. Int. J. Neuropsychopharmacol. 1–17.

Rotondo, D., Abril, H.T., Milton, A.S., Davidson, J., 2018. Pyrogenic immunomodulators increase the level of proinflammatory cytokines in patients with COVID-19. J. Neurol. Neurosurg. Psychiatry 97, 650–662.

Shujaa, N., Zadori, Z.S., Ronai, A.Z., Barna, I., Mergl, Z., Mozes, M.M., Gyires, K., 2009. Analysis of the effect of neuropeptides and cannabinoids in gastric mucosal defense initiated centrally in the rat. J. Physiol. Pharmacol. 60 (Suppl. 7), 93–100.

Skaper, S.D., Facci, L., Barbieri, M., Zonzo, M., Bruschetta, G., Impellizeri, D., Cuzzocrea, S., Giusti, P., 2015. N-Palmitoylethanolamide and Neuroinflammation: a novel therapeutic strategy of resolution. Mol. Neurobiol. 52, 1034–1042.

Soleimanpour, C., Zhu, G., Battista, N., Astari, G., Lodola, A., Rivera, P., Mur, R., Russo, R., Maccarrone, M., Antonietti, F., Duranti, A., Tontini, A., Cuzzocrea, S., Tarzia, G., Piemelli, D., 2009. Selective N-acylethanolamide-oxidizing acid amide inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. Proc. Natl. Acad. Sci. U. S. A. 106, 20966–20971.

Song, J.W., Kim, H.J., Lee, H., Kim, J.W., Kwak, Y.L., 2016. Protective effect of peroxisome proliferator-activated receptor-alpha activation against cardiac ischemia-reperfusion injury is related to Upregulation of Uncoupling Protein-3. Oxidative Med. Cell. Longev. 2016, 3539649.

Steiner, A.A., Ivanov, I.A., Serrats, J., Hosokawa, H., Phayre, A.N., Roberts, J.R., Kobayashi, S., Matsumura, K., Sawchenko, P.E., Romanovsky, A.A., 2006. Cellular and molecular bases of the initiation of fever. PLoS Biol. 4, e284.

Steiner, A.A., Molchanova, A.Y., Dogan, P.H., Patel, S., Wanner, S.M., Eales, J., Oliveira, D.L., Gavva, N.R., Almeida, M.C., Szekely, M., Romanovsky, A.A., 2011. The hypothermic response to bacterial lipopolysaccharide critically depends on brain CB1, but not CB2 or TRPV1, receptors. J. Physiol. 589, 2415–2431.

Tahamant, A., Takavoli-Yaraki, M., Rygiel, T.P., Mokhtari-Atazi, T., Salimi, V., 2016. Effects of cannabinoids and their receptors on viral infections in humans. J. Med. Virol. 88, 1–12.

Taquet, F., Sierra Luciano, S., Geddes, J.R., Harrison, P.J., 2021. Bidirectional associations between COVID-19 and psychiatric disorders: retrospective cohort studies of 62354 COVID-19 cases in the USA. Lancet Psychiatry 8, 130–140.

Walker, D.G., Tang, T.M., Lue, L.F., 2018. Increased expression of toll-like receptor 3, an anti-viral signalling molecule, and related genes in Alzheimer’s disease brains. Exp. Neurol. 309, 91–106.

Xu, X., Guo, H., Jing, Z., Yang, L., Chen, C., Peng, L., Wang, X., Yan, Y., Ye, J., Xin, J., Wang, Y., 2016. N-Oleoyl ethanolamide reduces inflammatory cytokines and activator receptor-alpha, alters spinal neuronal firing in a rodent model of neurogenic pain. Scand J Pain 9, 42–48.

Yorio, I., Ales, F., Pesaresi, B., Ferri, A., Serrano, A., Garcia-Bueno, B., 2018. Oleoylethanolamide, neuroinflammation, and alcohol abuse. Front. Mol. Neurosci. 11, 490.

Borges-Campana, S., Ferri, A., Aveta, T., Biento, L., Valaschi, G., Fiorenzani, P., Di Mauro, V., Orlando, P., Izzo, A.A., 2020. Protective effect of palmitoylethanolamide in a rat model of cystitis. J. Urol. 193, 1401–1408.

Petsalos, E., Alegro, A., Biron, M., Uskokovic, T., Gazi, T., De Filippis, D., Amanic, S., Saturnino, C., Orlando, P., Zimmer, A., Iovino, D., Di Mauro, V., 2010. Protective role of palmitoylethanolamide in contact allergic dermatitis. Allergy 65, 698–711.

Piemelli, D., Scalvini, L., Fotio, Y., Lodola, A., Spadoni, G., Tarzia, G., Mor, M., 2020. N-Acylethanolamine acid Amidase (NAAA): structure, function, and inhibition. J. Med. Chem. 63, 7475–7490.

Plaza-Zabala, A., Berrendero, F., Suarez, J., Bermudez-Silva, F.J., Fernandez-Espino, E., Serrano, A., Pavon, F.-J., Parsons, L.H., De Fonseca, F.R., Maldonado, R., Robledo, P., 2010. Effects of the endogenous PPAR-α agonist, oleoylethanolamide on MDMA-induced cognitive deficits in mice. Synapse 64, 379–389.

Rosner, M.R., 2021. Cannabidiol inhibits SARS-CoV-2 replication and promotes the host innate immune response. bioRxiv https://doi.org/10.1101/2021.03.04.429567.

Okada, R.N., Wood, C., Mills, P., Bennett, A., Chapman, V., 2015. Systemic administration of WY-14463, a selective synthetic agonist of peroxisome proliferator

possible strategy to treat mast cell-induced lung inflammation in COVID-19. Med. Hypotheses 143, 109856.

Gonzalez-Aparicio, R., Blanco, E., Serrano, A., Pavon, F.J., Parsons, L.H., Maldonado, R., Robledo, P., Fernandez-Espino, E., de Fonseca, F.R., 2014. The systemic administration of oleoylthanolamide exerts neuroprotection of the nigrostriatal system in experimental parkinsonism. J. Neurophysiol. 17, 455–468.

Harangozó, B.N., Voo, H.R., 2015. Neurological symptoms of mast cell activation syndrome complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19). J. Neurol. https://doi.org/10.1007/s00415-020-09602-9.
adhesion molecules in TNF-alpha-induced human umbilical vein endothelial cells by activating CB2 and PPAR-alpha. J. Cardiovasc. Pharmacol. 68, 280–291.

Yang, L., Guo, H., Li, Y., Meng, X., Yan, J., Dan, Z., Wu, S., Zhou, H., Peng, L., Xie, Q., Jin, X., 2016. Oleoylethanolamide exerts anti-inflammatory effects on LPS-induced THP-1 cells by enhancing PPARalpha signaling and inhibiting the NF-kappaB and ERK1/2/AP-1/STAT3 pathways. Sci. Rep. 6, 34611.

Zhou, Y., Yang, L., Ma, A., Zhang, X., Li, W., Yang, W., Chen, C., Jin, X., 2012. Orally administered oleoylethanolamide protects mice from focal cerebral ischemic injury by activating peroxisome proliferator-activated receptor alpha. Neuropharmacology 63, 242–249.