

## EDITORIAL

# Cannabinoids: clearing the smoke on pain, inflammation and neurodegeneration

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### LINKED ARTICLES

This article is part of a themed section on Cannabinoids 2013.

To view the other articles in this section, see Fagan and Campbell (2014) DOI: 10.1111/bph.12492, Ignatowska-Jankowska *et al.* (2014) DOI: 10.1111/bph.12298, Ligresti *et al.* (2014) DOI: 10.1111/bph.12410, Lopez-Rodriguez *et al.* (2014) DOI: 10.1111/bph.12519, Murataeva *et al.* (2014) DOI: 10.1111/bph.12411, Okine *et al.* (2014) DOI: 10.1111/bph.12540, Rodríguez-Cueto *et al.* (2014) DOI: 10.1111/bph.12283, Stanley and O'Sullivan (2014) DOI: 10.1111/bph.12560, Toguri *et al.* (2014) DOI: 10.1111/bph.12545, Wu *et al.* (2014) DOI: 10.1111/bph.12500.

Linked articles are: Avraham *et al.* (2014) DOI: 10.1111/bph.12478, Machado *et al.* (2014) DOI: 10.1111/bph.12488, Ward *et al.* (2014) DOI: 10.1111/bph.12439.

This themed section of BJP arises from the 6<sup>th</sup> European Workshop on Cannabinoid Research held in Dublin, Ireland from 18–20 April 2013. The section brings together 3 reviews and 10 research articles, presenting a range of work across the cannabinoid field.

The review article by Fagan *et al.* (2014) addresses the impact of endogenous, plant-derived and synthetic cannabinoids on neuronal viability. This review summarizes common mechanisms of neurodegeneration (including mitochondrial dysfunction, neuroinflammation and excitotoxicity) in three prominent age-related neurodegenerative disorders, Alzheimers' disease, Parkinson's disease and Huntington's disease. The review focuses on literature highlighting the cannabinoid system as a bona fide therapeutic target against such neurodegenerative processes. In addition, this review brings us up-to-date on the close links between the endocannabinoid system, ageing and the process of adult neurogenesis.

Stanley and O'Sullivan (2014) review the vascular targets of cannabinoids. They examine the evidence for the range of receptors activated by cannabinoids in perfused vascular beds

or isolated arteries. Direct targets of synthetic, plant-derived or endogenous cannabinoids include CB<sub>1</sub>, CB<sub>2</sub>, TRPV1, peroxisome proliferator-activated receptors (PPARs), CB<sub>2</sub>, GPR55 and 5-HT<sub>1A</sub> receptors. Prostanoid receptors and the calcitonin gene-related peptide (CGRP) receptor can also indirectly mediate the vascular response to cannabinoids. Typically cannabinoids have vasorelaxant effects, although vasoconstriction has also been observed in some instances. Animal and human studies are reviewed.

The third review article discusses one of the most abundant endocannabinoids, 2-arachidonylglycerol (2-AG), and summarizes the latest major discoveries associated with the complex pathways leading to 2-AG synthesis and metabolism in the central nervous system (CNS) (Murataeva *et al.*, 2014). This review addresses the spatial and temporal expression of diacylglycerol lipases (DAGL) in the CNS, the most heavily studied pathway for 2-AG synthesis. Intriguing questions regarding 2-AG metabolizing enzymes are considered, including their activity in different cellular compartments and their contribution to endogenous 2-AG-based signalling. The

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review highlights that the various enzymes for synthesizing and degrading 2-AG represent potential therapeutic targets for neurodegenerative and inflammatory diseases.

Research on the function of 2-AG has been facilitated in recent years by the availability of some selective and potent inhibitors of the catabolising enzyme monoacylglycerol lipase (MAGL). One such inhibitor is the compound KML29 and Ignatowska-Jankowska *et al.* (2014) present novel data herein on the effects of this MAGL inhibitor in mouse models of inflammatory (carrageenan) and neuropathic (sciatic nerve injury) pain. Their results show that systemic administration of KML29 attenuates carrageenan-induced paw oedema and reverses carrageenan-induced mechanical allodynia. Tolerance to these effects of KML29 and CB<sub>1</sub> receptor desensitization developed after repeated administration of high doses. Moreover, KML29 partially reversed sciatic nerve injury-induced allodynia and prevented diclofenac-induced gastric haemorrhages, without eliciting cannabimimetic effects in the tetrad test. These preclinical data suggest that MAGL inhibitors show promise as novel analgesics with a favourable side-effect profile.

An original article from the laboratory of Vincenzo Di Marzo, in collaboration with colleagues in Selcia UK and Allergan USA, describes the results of pharmacological screening of a series of receptor antagonists for prostamides, neutral lipid mediators produced following oxidation of the endocannabinoid, anandamide (Ligresti *et al.*, 2014). Endogenous prostamide F<sub>2α</sub> exhibits pro-nociceptive properties, suggesting that prostamide F<sub>2α</sub> receptor antagonists may be of use in the treatment of pain. In their studies, the authors targeted fatty acid amide hydrolase (FAAH), the main hydrolytic enzyme catalyzing the degradation of anandamide, using rat, mouse and human tissues. The findings indicate that synthetic prostamide F<sub>2α</sub> receptor antagonists AGN 211335 and AGN 211336 inhibit FAAH in various species *in vitro*, and furthermore demonstrate that AGN 211335 has the proclivity to weakly bind human recombinant CB<sub>1</sub> receptors. The anti-nociceptive actions of AGN 211335 and AGN 211336 were validated *in vivo* in the formalin model of inflammatory pain. The authors conclude that prostamide F<sub>2α</sub> antagonists may act as both FAAH inhibitors and prostamide receptor blockers, with further investigation warranted to evaluate these compounds in chronic inflammation.

While FAAH inhibitors clearly demonstrate potential as novel therapeutic agents for a range of disorders, the effects of perinatal exposure to FAAH inhibitors on brain development and behaviour in adulthood are under-studied. In this themed section, Wu and colleagues have addressed this question in their paper entitled 'Long-term consequences of perinatal fatty acid amino hydrolase inhibition' (Wu *et al.*, 2014). Daily treatment of mouse dams with the FAAH inhibitor URB597 (1, 3 or 10 mg kg<sup>-1</sup>) from gestational day 10.5 to 16.5 did not induce toxicity or weight gain in dams, nor did it affect neurogenesis or axonal development in 16.5 day-old embryos. In additional work, treatment of mouse dams with URB597 from gestational day 10.5 to postnatal day 7 resulted in reduced cocaine-conditioned preference, increased depressive behaviour in the forced swim test, and impaired working memory in the offspring at 2–4 months of age. These novel data suggest that exposure to elevated

levels of anandamide and related *N*-acyl amides during *in utero* development may lead to subtle behavioural alterations in adulthood.

Continuing with the theme of the impact of early-life exposure to cannabinoids, Lopez-Rodriguez *et al.* (2014) show that there are long-term effects of adolescent exposure to Δ<sup>9</sup>-tetrahydrocannabinol (THC), with or without methylenedioxymethamphetamine (MDMA, 'Ecstasy'), on neuroinflammation and on the serotonergic and endocannabinoid systems in rats. Specifically, they have demonstrated that chronic treatment with THC during adolescence increases expression of glial fibrillary acidic protein (GFAP) in the hippocampus of both male and female rats. However, differential effects of either THC or MDMA administration on the percentage of reactive microglia in the hippocampus of male versus female rats were observed. Moreover, in male rats, THC decreased the number of SERT+ fibres in the parietal cortex, while MDMA had the opposite effect. THC administration resulted in reduced expression of the CB<sub>1</sub> receptor in the hippocampus, an effect that was potentiated by MDMA co-administration.

In a recent issue, Avraham *et al.* (2014) looked at the neurogenetic role of the CB<sub>2</sub> receptor agonist AM1241 in the GFAP/Gp120 transgenic (Tg) mouse model of HIV-1 infection. This model recapitulates some of the cognitive deficits seen in patients with HIV-1, and is associated with astrogliosis, neuronal degeneration and deficits in hippocampal neurogenesis in the brains of these mice. The authors demonstrate that AM1241 robustly inhibits Gp120-induced apoptosis in primary murine and human neural progenitor cells. Furthermore, administration of AM1241 to GFAP/Gp120 Tg mice resulted in both enhanced hippocampal neurogenesis and a reduction in the number of reactive astrocytes in the Sub-Granular Zone *in vivo*. The implications of these findings include the possibility that CB<sub>2</sub> receptor-specific agonists may act as neuroprotective agents for rescuing impaired neurogenesis in patients with HIV-associated dementia.

Toguri *et al.*, (2014) investigated the immunomodulatory effects of a CB<sub>2</sub> receptor agonist (HU308) and antagonist (AM630) using *in vivo* real-time imaging and *ex vivo* analysis of inflammatory markers in an acute model of experimental endotoxin-induced uveitis (EIU). This particular report demonstrates that CB<sub>2</sub> receptor activation is anti-inflammatory in the eye. The authors clearly demonstrate that CB<sub>2</sub> receptor-mediated anti-inflammatory effects are mediated by a decrease in the transcription factors NF-κβ and AP-1 with resultant reduction in cytokines, chemokines and adhesion molecules. Importantly, the anti-inflammatory actions of CB<sub>2</sub> receptor modulation in this model were more efficacious than clinically relevant treatments for uveitis, indicating that CB<sub>2</sub> receptor-specific agonists may act as novel ocular anti-inflammatory drug targets.

The contribution from Machado *et al.* (2014) assessed cross-talk between the cannabinoid and opioid systems in pain management. The authors investigated the cannabinoid-mediated anti-nociceptive activity of crotalphine, a synthetic peptide with opioid-like activity, following induction of hyperalgesia in rats via intraplantar administration of prostaglandin E<sub>2</sub>. Crotalphine promoted CB<sub>2</sub> receptor activation in paw tissue, and blockade of CB<sub>2</sub>, using the antagonist AM630, prevented the anti-nociceptive activity of

crotalphine. In addition, this effect of crotalphine was mediated by the CB<sub>2</sub> receptor-dependent release of dynorphin A, the endogenous agonist of *kappa* opioid receptors. The authors conclude that their findings support the close connection between the opioid and cannabinoid systems in the control of pain pathways.

In another interesting manuscript focused on the effects of cannabinoids on pain, Ward *et al.* (2014), present novel data on the effects of cannabidiol on paclitaxel-induced neuropathic pain-related behaviour. Chemotherapeutic agents such as paclitaxel are thought to induce neuropathic pain in a significant number of patients and this adverse effect often limits their usefulness. The paper by Ward and colleagues demonstrated that sub-chronic dosing with cannabidiol prevents the development of paclitaxel-induced mechanical hypersensitivity in mice. Furthermore, this effect was blocked by co-administration of the 5-HT<sub>1A</sub> receptor antagonist WAY 100635, but not by CB<sub>1</sub> or CB<sub>2</sub> receptor antagonists. Place conditioning and autoshaping were also studied and were found to be unaffected by cannabidiol treatment suggesting that this cannabinoid had no rewarding effects and did not affect learning and memory in these paradigms. Combinations of paclitaxel and cannabidiol were found to produce additive to synergistic inhibition of breast cancer cell viability. These data support additional recent reports of the efficacy of cannabinoids and endocannabinoid system modulators in animal models of chemotherapy-induced neuropathic pain (Deng *et al.*, 2012; Guindon *et al.*, 2013).

Focusing on supraspinal regulation of inflammatory pain, Okine *et al.* (2014), published herein, presents novel data on the effects of direct administration of a selective PPAR- $\alpha$  agonist and antagonist into the medial prefrontal cortex (mPFC) on formalin-evoked nociceptive behaviour in rats. The results demonstrate that intra-mPFC administration of the PPAR- $\alpha$  antagonist GW6471 delayed the onset of second phase formalin-evoked nociceptive behaviour while the PPAR- $\alpha$  agonist GW7647 had no effect. Formalin-evoked nociceptive behaviour was associated with significant reductions in mPFC levels of endogenous PPAR- $\alpha$  ligands (*N*-palmitoylethanolamide [PEA] and *N*-oleoylethanolamide [OEA]) and a 70% reduction in PPAR- $\alpha$  mRNA. These data suggest that PPAR- $\alpha$  in the mPFC may play a facilitatory/permissive role in formalin-evoked nociceptive behaviour in rats. Thus, supraspinal PPARs represent a non-CB<sub>1</sub>/CB<sub>2</sub> target for endocannabinoids and related *N*-acylethanolamines with potential as a novel therapeutic target for inflammatory pain.

Spinocerebellar ataxias are a family of chronic progressive neurodegenerative diseases characterized by loss of balance and motor coordination due to degeneration of the cerebellum and its afferent and efferent connections. Using immunohistochemistry, Rodríguez-Cueto *et al.* (2014) show that levels of CB<sub>1</sub> and CB<sub>2</sub> receptor expression are higher in granular layer, Purkinje cells, dentate nucleus and areas of white matter in the post-mortem cerebellum of spinocerebellar ataxia patients, compared with controls. Further immunohistochemistry confirmed that the presence of CB<sub>1</sub> and CB<sub>2</sub> receptor in Purkinje neurons, as well as in microglia and astrocytes. Thus, the endocannabinoid system represents a potential therapeutic target for the treatment of spinocerebellar ataxias.

Overall then, the cannabinoid field remains very vibrant. The 'old favourites' CB<sub>1</sub> and CB<sub>2</sub> are still the subject of much research but non-CB<sub>1</sub>/CB<sub>2</sub> targets for endocannabinoids, and other components of the endocannabinoid system, have become a keen focus for many laboratories interested in pain, inflammation and neurodegeneration. Sir William B. O'Shaughnessy was born in Limerick, Ireland in 1809 and has been credited as the first person to introduce cannabis to Western medicine. The work presented at the 6<sup>th</sup> European Workshop on Cannabinoid Research held in his native country in April 2013, and that presented in this themed section, highlights the tremendous progress that has been made in the area of cannabinoid pharmacology and the potential of the endocannabinoid system as a promising therapeutic target for a wide range of disorders.

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