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Review

Non-psychotropic analgesic drugs from the endocannabinoid system: "Magic bullet" or "multiple-target" strategies?

Q1 Katarzyna Starowicz [a,*], Vincenzo Di Marzo [b]

[a] Department of Pain Pharmacolgy, Institute of Pharmacology Polish Academy of Sciences, 12 Smetna str, 31-343 Krakow, Poland
[b] Endocannabinoid Research Group, Institute of Biomolecular Chemistry, National Research Council, Via Campi Flegrei 34, 80078 Pozzuoli, Naples, Italy

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ABSTRACT

The exploitation of preparations of *Cannabis sativa* to combat pain seems to date back to time immemorial, although their psychotropic effects, which are at the bases of their recreational use and limit their therapeutic use, are at least as ancient. Indeed, it has always been different to tease apart the unwanted central effects from the therapeutic benefits of Δ^9-tetrahydrocannabinol (THC), the main psychotropic component of cannabis. The discovery of the cannabinoid receptors and of their endogenous ligands, the endocannabinoids, which, unlike THC, play a pro-homeostatic function in a tissue- and time-selective manner, offered the opportunity to develop new analgesics from synthetic inhibitors of endocannabinoid inactivation. The advantages of this approach over direct activation of cannabinoid receptors as a therapeutic strategy against neuropathic and inflammatory pain are discussed here along with its potential complications. These latter have been such that clinical success has been achieved so far more rapidly with naturally occurring THC or endocannabinoid structural analogues acting at a plethora of cannabinoid-related and -unrelated molecular targets, than with selective inhibitors of endocannabinoid enzymatic hydrolysis, thus leading to revisit the potential usefulness of "multi-target" versus "magic bullet" compounds as new analgesics.

1. Introduction

The medicinal properties of the plant *Cannabis sativa* were first recorded in 2737 BC in Shen Nung dynasty China, and it were well known and employed by physicians also in Victorian Britain, for pain relief (analgesia) or decreased pain sensitivity (antinociception), which are among the most commonly cited therapeutic effects of smoked *Cannabis*. However, the detailed description and the discovery of the evolutionary ancient signaling system using the same receptors as the psychotropic and analgesic constituents of *Cannabis*, has taken a relatively long time. The initial step toward the discovery of this "endocannabinoid system" was the finding of the chemical identity of the principal psychoactive constituent of *Cannabis*, Δ^9-tetrahydrocannabinol (THC) to Gaoni and Mechoulam (1964). Subsequently, Howlett and colleagues discovered the THC binding sites in the brain (Devane et al., 1988), thus leading, a few years later, to the cloning of cannabinoid receptors of type-1 (CB_1) and -2 (CB_2) (Matsuda et al., 1990; Munro et al., 1993). Another important step was the development of pharmacological tools manipulating cannabinoid receptor function and the development of CB_1 and CB_2 knock-out mice, concomitantly with the identification of the first endocannabinoid, *N*-arachidonylethanolamide or anandamide (AEA) (Devane et al., 1992), which opened the way later to finding a second endocannabinoid 2-arachidonylglycerol (2-AG), Mechoulam et al. (1995). Finally, the discovery that AEA binds not only to CB receptors but also other targets, such as transient receptor potential vanilloid 1 channel (TRPV1) (Zygmunt et al., 1999) was an important milestone in endocannabinoid research.

Endocannabinoids are not only chemically but also functionally different form THC. Endogenous agonists of CB_1 and CB_2 receptors, act as local chemical mediators, synthesized "on demand" (van der Stelt et al., 2005). Unlike hormones or neuropeptides, endocannabinoids act in an autocrine or paracrine manner and are immediately metabolized. Enzymes for endocannabinoid biosynthesis from preformed membrane lipids and phospholipids and endocannabinoid oxidation (by several enzymes of the arachidonate cascade) and, more often, hydrolysis have been identified and characterized.

The endocannabinoid system regulates many aspects of health with receptors located throughout the body including the central and peripheral nervous systems. The CB_1 receptor is expressed most abundantly in the brain, but is also present in peripheral tissues, including the lungs, liver, kidneys and adipose tissue (Pacher et al., 2006). The CB_2 receptor is mainly expressed in the

* Corresponding author. Tel.: +48 12 6623240; fax: +48 12 6374500.
E-mail addresses: starow@if-pan.krakow.pl (K. Starowicz), vdimarzo@icmib.na.cnr.it (V. Di Marzo).

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immune system and in hematopoietic cells, but may be induced during several pathological conditions also in other tissues and cells (Pacher et al., 2006; Racz et al., 2008). Activation of CB_1 receptors increases reward; reduce pain, anxiety, body temperature, blood pressure; stimulate food consumption; inhibit motor behaviors, induce sedation; mediate extinction of aversive memories and fear. Endocannabinoid also have neuroprotective and anti-inflammatory functions (Mechoulam and Parker, 2012). The discovery of the endocannabinoid system has transformed preclinical research on pain, and led to a greater understanding of its brain and spinal aspects. The widespread distribution of CB receptors in the pain processing pathways encourages its potential for analgesia. Endocannabinoids have been shown to be involved in the control of pain both at the level of ascending pathways, from the sensory nerves to the brain, and of the descending pain inhibitory pathways that provide negative feedback control of nociceptive signals at the spinal cord level. Thus endocannabinoids inhibit pain at the peripheral, spinal and supraspinal levels (Manzanares et al., 2006). The administration of exogenous cannabinoids and cannabis-based medicines raises safety concerns for patients. Cannabinoids acting on a specific receptor that is widely distributed in brain regions involved in cognition, memory, reward, and motor coordination, that is the CB_1 receptors, produce efficacious analgesic actions but also evoke therapeutically undesirable psychotropic effects. On the other hand, agents that selectively target CB_2 receptors, although still efficacious at reducing inflammatory and chronic pain, and generally non-psychoactive, may produce immune depression and have yielded thus far disappointing results in clinical trials (Atwood et al., 2012). Therefore, the discovery of the endocannabinoid system and of endocannabinoid -degrading enzymes offers the opportunity to develop drugs against inflammatory and chronic pain potentially safer that CB_1 or CB_2 exogenous agonists. In fact, since during pain endocannabinoids are produced and degraded selectively only in tissues participating in pain control, such drugs, by elevating endocannabinoid levels only locally, might lead to indirect activation of CB receptors only in these tissues.

Indeed, selective targeting of endocannabinoid-degrading enzymes is a promising strategy to treat pain syndromes. However, endocannabinoids, and AEA in particular, may interact with other targets, and produce, as in case of TRPV1 activation, pronociceptive effects. Furthermore, the existence of multiple endocannabinoid -degradation pathways may minimize the impact of this strategy to elevate endocannabinoid levels and activate CB receptors indirectly, since by inhibiting one enzyme alterative degradation pathway(s) may become activated. Consequently, this strategy may promote the formation of other molecules active at different molecular targets, again with opposite function to CB receptor activation (Petrosino and Di Marzo, 2010). In the light of these possible complications, an interesting approach to benefit from the body's endocannabinoid system for pain relief relies on the development of multi-targetmodulators. The polypharmacology approach targeting both endocannabinoid break-down mechanisms and other possible endocannabinoid molecular targets, which may become activated upon the increase in endocannabinoid levels, may afford higher efficacy with lower or no side effects.

2. Endocannabinoid-based analgesic drugs: Inhibitors of endocannabinoid enzymatic hydrolysis

Recent studies have investigated the targeting of the endocannabinoids, rather than the cannabinoids receptors, as an alternative approach to achieve analgesia in the absence of central side effects. While exogenously administered endocannabinoids are rapidly degraded by catabolic enzymes, i.e.fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) for AEA and 2-AG, respectively, pharmacological inhibition of these enzymes results in elevated endocannabinoid levels in brain and spinal cord tissues (Cravatt et al., 1996; Kinsey et al., 2009; Long et al., 2009). Mice lacking FAAH possess highly elevated endogenous levels of AEA and other fatty acid amides in several brain regions (Clement et al., 2003) and display CB_1-dependent analgesia (Cravatt et al., 2001; Lichtman et al., 2004). These data confirm the key role that FAAH plays in regulating fatty acid amide signaling *in vivo* and suggest that this enzyme may represent an attractive target for the treatment of pain. In the case of 2-AG, pharmacological inhibition or genetic inactivation of MAGL do not necessarily lead to similar effects on nociception (see below), but, again it has been demonstrated that this enzyme plays a major role in controlling cannabinoid receptor "tone". Some of the most promising results documenting the potential therapeutic effects of FAAH and MAGL inhibition are summarized in Table 1.

2.1. FAAH blockade and pain

Blockade of FAAH leads to a hypoalgesic phenotype in several laboratory animals models of nociception (Lichtman et al., 2004a). Potent and selective reversible FAAH inhibitors were demonstrated to exhibit selectivity for FAAH compared with previously described inhibitors, to augment the endogenous levels of fatty acid amides in the central nervous system, and to produce CB_1-dependent analgesic effects in both thermal and chemical pain models (Lichtman et al., 2004a). The availability of FAAH −/− mice, which exhibit a profound reduction in hydrolysis activity for anandamide and other FAAs (Cravatt et al., 2001), provided a powerful model to investigate the function of FAA signaling pathways. FAAH −/− mice exhibited a CB_1 receptor-mediated phenotypic hypoalgesia in thermal nociceptive tests and CB_1 receptor-mediated hypoalgesia in both phases of the formalin test accompanied with a phenotypic anti-edema effect, which, instead, was not blocked by either CB_1 or CB_2 antagonists. Additionally, FAAH −/− mice displayed thermal anti-hyperalgesic and anti-inflammatory effects in the carrageenan model, which were mediated, in part, by CB_2, but not CB1 receptors. In contrast, no genotypic differences in pain behavior were evident in models of chronic pain, which was instead found to eliminate the phenotypic hypoalgesia displayed by FAAH −/− mice (Lichtman et al., 2004b), suggesting that nerve injury may promote adaptive changes in these animals and that the role or the extent of the participation of the enzyme and its substrates in chronic pain is lesser than in the acute or inflammatory pain states. For example, the elevation in AEA levels and/or other FAAs in FAAH −/− mice may have been insufficient to block thermal hyperalgesia in the chronic constriction injury (CCI) model. Alternatively, nerve ligation may have led to adaptive changes in the nociceptive circuits of FAAH −/− mice that reduce the influence of endogenous FAAs over pain behavior (Lichtman et al., 2004b). Therefore, FAAH inhibition rather than deletion may offer a distinctive strategy for the treatment of chronic pain disorders.

Indeed, both irreversible (i.e. [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate, URB597) and reversible (i.e. α-ketoheterocycle, OL-135)FAAH inhibitorsreduce nociceptive responses in acute (Holt et al., 2005; Naidu et al., 2009, 2010), and chronic models of pain (Jayamanne et al., 2006). These effects have been attributed to endocannabinoid-mediated activation of cannabinoid receptors (Jayamanne et al., 2006; Lichtman et al., 2004a). In the CFA-induced model of inflammation,URB597 effects were attenuated by CB_1 and CB_2 cannabinoid receptor antagonists (Jayamanne et al., 2006). In the carrageenan-inflamed hindpaw model, instead, although both tested doses of URB597 increased levels of AEA

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