HHS Public Access

Author manuscript

Pain. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Pain. 2021 July 01; 162(Suppl 1): S5–S25. doi:10.1097/j.pain.0000000000002268.

Cannabinoids, the endocannabinoid system and pain: a review of preclinical studies

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Abstract

This narrative review represents an output from the International Association for the Study of Pain's global task force on the use of cannabis, cannabinoids, and cannabis-based medicines (CBM) for pain management, informed by our companion systematic review and meta-analysis of preclinical studies in this area. Our aims in this review are: 1) to describe the value of studying cannabinoids and endogenous cannabinoid (endocannabinoid) system modulators in preclinical/animal models of pain; 2) to discuss both pain-related efficacy and additional pain-relevant effects (adverse and beneficial) of cannabinoids and endocannabinoid system modulators as they pertain to animal models of pathological or injury-related persistent pain; and 3) to identify important directions for future research. In service of these goals, this review a) provides an overview of the endocannabinoid system and the pharmacology of cannabinoids and endocannabinoid system modulators, with specific relevance to animal models of pathological or injury-related persistent pain; b) describes pharmacokinetics of cannabinoids in rodents and humans; and c) highlights differences and discrepancies between preclinical and clinical studies in this area. Preclinical (rodent) models have advanced our understanding of the underlying sites and mechanisms of action of cannabinoids and the endocannabinoid system in suppressing nociceptive signaling and behaviors. We conclude that substantial evidence from animal models supports the contention that cannabinoids and endocannabinoid system modulators hold considerable promise for analgesic

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drug development, although the challenge of translating this knowledge into clinically useful medicines is not to be underestimated.

Keywords

Cannabinoid$_1$ (CB_1) receptor; Cannabinoid$_2$ (CB_2) receptor; Endocannabinoid; Chronic Pain; Neuropathic Pain; Inflammatory Pain; Nociception; rats; mice; behavior

Introduction

Cannabis, cannabis extracts or oils, individual cannabinoid substances, and modulators of the endogenous cannabinoid (endocannabinoid) system have all been suggested as therapeutic agents for pain management[75,94,193]. The primary drivers for interest in these agents include: 1) major scientific advances in our understanding of the biology of the endocannabinoid system and pharmacology of cannabinoids; 2) development and regulatory approval of cannabis-based (or cannabis-derived) medicines (e.g. Nabiximols/ Sativex® and Epidiolex®); and 3) regulatory changes that permit use of cannabis for medical purposes, including pain management, following advocacy by patients and public support. However, the latter development has proven controversial amongst scientists, patients and healthcare professionals, with uncertainty over whether the current evidence base for efficacy and safety justifies change in legislation of cannabis for medical use, including pain management. In 2018, the International Association for Study of Pain (IASP) established a Presidential Task Force on Cannabis and Cannabinoids Analgesia. The present narrative review represents an output from Work Package (WP) 1 of the IASP task force which was focused on basic science, including definition of terminology, overview of the endogenous cannabinoid (endocannabinoid) system, compound classification, pharmacology and assessment of pain-related efficacy and additional pain-relevant effects (adverse and beneficial) in preclinical laboratory animal studies.

The aims of this review are:

1. To provide commentary on the efficacy and side effects of cannabinoids and endocannabinoid system modulators as they pertain to animal models of pathological pain[a], informed by our companion systematic review and meta-analysis of preclinical studies in this area[215].
2. To discuss the value of studying cannabinoids and endocannabinoid system modulators in preclinical/animal models of pain, as well as discrepancies between preclinical and clinical studies in this area, and
3. Provide suggestions for future research directions.

In service of the above objectives, we:

a. Clearly define terminology used in this field.

[a]The term ‘animal model of pain’ is not a universally agreed descriptor, but given that it is common usage we will use it herein as shorthand. It is also worth noting that there is a distinction between ‘model’ which reflects the underlying disease or injury and the pain-associated outcome measures used in evaluating such models.

b. Provide an overview of the endocannabinoid system.

c. Review the classification, chemistry, and pharmacology of cannabinoids, CBM and endocannabinoid system modulators, including structure-activity relationships and pharmacokinetics in rodents and humans.

Terminology and Definitions

One factor that has hampered public debate (and sometimes scientific/medical debate) on the topic of cannabis and cannabinoids for pain management is the inappropriate, inconsistent or unclear use of terminology. For example the terms ‘cannabis’, ‘cannabinoids’ and ‘cannabis-based medicines (CBM)’ are often used interchangeably or conflated, both within public discourse and the media, and within the scientific literature (e.g. pooling of data relating to cannabis and individual cannabinoids in meta-analyses and systematic reviews of clinical efficacy). Cannabis refers to the whole plant, or to its parts, and must be clearly distinguished from cannabinoid ligands (cannabinoids) that are either plant-derived natural (phytocannabinoids), synthetic or semi-synthetic, but always chemically defined, single entity compounds usually having affinity for and activity at cannabinoid receptors. Thus, cannabis, single entity cannabinoid compounds, and modulators of the endocannabinoid system should not be used synonymously or conflated within the same preclinical or clinical investigation for either efficacy or side effects. Furthermore, when considering or debating the merits, or otherwise, of cannabis, due regard must be given to the fact that cannabis is highly heterogeneous with many different chemical constituents and that a multitude of different strains of the plant exist, all containing different amounts of phytocannabinoids[12,103], most particularly Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC, first isolated in the 1960s, is the primary psychoactive constituent of *Cannabis sativa*[83,162]. THC has psychoactive properties, and its pharmacological effects are attributable to agonist activity at both cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors[44,61,148,234]. CBD, in contrast, does not have appreciable agonist activity at CB_1 receptors (but may be a negative allosteric modulator of CB_1), and lacks the psychoactivity profile of THC[22,68,222]. In addition to phytocannabinoids, the cannabis plant also contains a large number of terpenes, flavonoids and other compounds, which, themselves, may be pharmacologically active but remain understudied.

CBMs are registered medicinal cannabis extracts, approved by regulatory authorities, with well-defined, standardized THC/CBD content, and with no, or only trace levels of, terpenes, flavonoids and other compounds. Examples of CBMs are nabiximols (Sativex®), approved in some countries for adjunctive treatment of spasticity (and in some jurisdictions neuropathic pain) in multiple sclerosis, and cannabidiol extract (Epidiolex®), indicated for treatment of childhood epilepsy, that has only minor or trace levels of other phytocannabinoids. These medicinal products with regulatory approval should be distinguished from so-called cannabis or CBD oils or extracts which are numerous, typically sold in healthfood stores, pharmacies, cannabis dispensaries or over the internet, but often have uncertain and/or unverified THC/CBD content. Synthetic THC (e.g. dronabinol), and the synthetic THC analogue nabilone are also approved for indications such as anorexia and weight loss in AIDS, and chemotherapy-induced nausea and vomiting, and available

on special prescription in many countries. Table 1 provides a glossary of terminology and definitions.

The Endocannabinoid System

The discovery of THC in the 1960s as the psychoactive constituent of *Cannabis* inspired research into its pharmacology and mechanism of action. However, it was not until the late 1980s/early 1990s that the cannabinoid receptors were discovered (Figure 1). The first cannabinoid receptor to be discovered, cloned and characterised was the CB_1 receptor, initially in rat brain[57,159] and subsequently localized in human brain[88]. In 1993, a second cannabinoid receptor, CB_2, was cloned and characterized in a human promyelocytic leukaemic cell line and in rat spleen[167]. Both CB_1 and CB_2 belong to the super-family of seven-transmembrane domain, G protein-coupled receptors (Gi/o coupled). The existence of cannabinoid receptors, highly conserved across species, implied the existence of endogenous cannabis-like molecules (endocannabinoids) that bind to and modulate cannabinoid receptors.

Research efforts sought to identify the endogenous ligands that bind to and modulate mammalian cannabinoid receptors. Two endocannabinoids, *N*-arachidonoylethanolamide (anandamide, AEA)[58] and 2-arachidonoyl glycerol (2-AG)[161,220], were discovered. The endocannabinoid system is an important physiological system, comprised of CB_1 and CB_2 receptors, their endogenous ligands, AEA and 2-AG, and the enzymes responsible for the synthesis and degradation of the endocannabinoids. AEA and 2-AG are the best characterised endocannabinoids. However, there are several other endogenous ligands with affinity and activity at cannabinoid receptors, including 2-AG ether (noladin ether), virodhamine, *N*-arachidonoyl dopamine (NADA) and others[10,59,63,191,192].

CB_1 is the most highly expressed G protein-coupled receptor subtype in the central nervous system (CNS)[88,104,191]. Within the brain, CB_1 is found in high density in the basal ganglia, as well as in key components of the descending pain pathway and the stress/fear/anxiety circuitry. CB_1 is localized to most other tissues and organs of the body. Of relevance to pain, in addition to their supraspinal localisation, CB_1 receptors are expressed in the dorsal horn of the spinal cord, synthesized in dorsal root ganglion cells and transported in peripheral nerves[21,74,108–111,178]. Immunohistochemical studies have localized CB_1 to dorsal root ganglia and identified CB_1 receptors on primary afferent neurons[21,110,111]. CB_2 receptors are mainly expressed in the periphery, with particularly high density on cells and tissues of the immune system[11,167]. Although localisation of CB_2 to otherwise naïve CNS remains controversial, CB_2 can be induced in the CNS in response to injury or pathophysiological states (for review see [99,134,205,240]). Being $G_{i/o}$ protein-coupled receptors, CB_1 and CB_2 are negatively coupled to adenylyl cyclase[116–118], and positively coupled to mitogen-activated protein kinase[18]. Within neurons, CB_1 activation results in inhibition of N- and P/Q-type voltage-activated Ca^{2+} channels, and induction of inwardly rectifying K^+ currents, with consequent inhibition of neurotransmitter release[55]. Cannabinoids, including endocannabinoids, phytocannabinoids, and synthetic cannabinoids may potentially also act at other non-CB_1/non-CB_2 receptors, including the transient receptor potential cation channel subfamily V member 1 (TRPV1; also known as the capsaicin or vanilloid receptor

VR1), peroxisome proliferator-activated receptors (PPARs), and G protein-coupled receptors such as GPR55 and GPR119[6,23,179].

Mechanisms underlying endocannabinoid biosynthesis, signaling and degradation are quite well understood. Biosynthesis of AEA involves its formation from the precursor N-arachidonoylphosphatidylethanolamine (NAPE), catalysed by the hydrolytic activity of the phospholipase D enzyme known as NAPE-PLD (for review see[13,30,31,62]). 2-AG is synthesized by conversion of 1,2-diacylglycerol to 2-AG by diacylglycerol lipases (DAGL) (for review see[59,135,219,227]). AEA is primarily degraded to arachidonic acid and ethanolamine by the enzyme fatty acid amide hydrolase (FAAH), located in the endoplasmic reticulum of postsynaptic neuron[46,59]for review see[86,182]. FAAH also catabolizes other *N*-acylethanolamines including *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA) which themselves do not have appreciable activity at CB_1 or CB_2 receptors but may elevate levels of AEA through substrate competition at FAAH[133]. 2-AG is primarily metabolized to arachidonic acid and glycerol by the presynaptic enzyme monoacylglycerol lipase (MGL)[65,227], with other enzymes including FAAH, ABHD6 and ABHD12 accounting for a modest (i.e. <10%) degree of 2-AG catabolism[15,89]. Within the nervous system, newly synthesized endocannabinoids leave the post-synaptic neuron to exert their effects on CB_1 receptors expressed on pre-synaptic nerve terminals in a signaling process known as retrograde neurotransmission. Further work is required to better understand the mechanisms by which endocannabinoids are transported within cells and across cell membranes.

As a lipid signalling system whose components are expressed widely across the body, the endocannabinoid system plays a key role in the regulation of a wide array of physiological processes including metabolism, mood, motor function, appetite, cardiovascular control, stress response, gastrointestinal tract function, developmental biology, cell fate, immune and inflammatory response, endocrine function, neurotransmission, and pain (for review see [60,183,184]). In the context of nociception and pain, key components of the endocannabinoid system are expressed throughout nociceptive pathways (Figure 2): in the periphery on primary afferent neurons, in the dorsal horn of the spinal cord and in multiple supraspinal regions of the brain associated with pain perception and modulation (for review see [96,99,196,207,217,240]). As a result, targeting the endocannabinoid system via enhancement of the levels of endogenous cannabinoids (e.g. with FAAH or MGL inhibitors) or exogenous cannabinoid ligands (e.g. CB_1 or CB_2 receptor agonists) can reduce nociceptive transmission at all three of these neuroanatomical levels. Glial cells, which express components of the endocannabinoid system, represent another substrate though which cannabinoids or endocannabinoid system modulators may regulate pain through neuro-immune interactions[29,157]for review see[233,238,242]. Preclinical research indicates that endocannabinoids are synthesized on-demand in postsynaptic neurons in response to stress or pain and produce short-term antinociceptive effects via presynaptic inhibitory CB_1 receptors[92,113,144,181,218,239]. Endocannabinoids are implicated in control of pain initiation[32,38], and play an important role in the resolution of tonic pain and in stress-induced and fear-conditioned analgesia in rodents[27,28,77,92,113]. In animal models of pathological pain, the endocannabinoid system exhibits adaptive changes or plasticity (e.g. altered cannabinoid receptor expression/functionality and endocannabinoid levels) depending on the model and anatomical site under investigation[100,200,206,240,241]. These

findings support the contention that the endocannabinoid system may represent a viable therapeutic target for chronic pain. This view is supported by numerous pharmacological studies demonstrating efficacy of cannabinoids or modulators of the endocannabinoid system in animal models of pathological or injury-related pain. These latter studies have been reviewed and analysed in our systematic review and meta-analysis of the preclinical literature published in this special issue of PAIN[215]. Herein, we include a summary of the key findings of this systematic review, and extended commentary on some elements not discussed in detail within the systematic review itself.

Classification of cannabinoids and endocannabinoid system modulators of relevance to pain

Common pharmacological tools employed to manipulate the endocannabinoid system are summarized in Table 2.

Antinociceptive efficacy of cannabinoids in animal models of pathological or injury-related persistent pain

The antinociceptive efficacy of cannabinoids and endocannabinoid system modulators has been reviewed extensively[99,196,206,217,240,241]. Thus, herein, we instead provide a concise commentary on the efficacy of cannabinoids and endocannabinoid system modulators in animal models of pathological or injury-related persistent pain that is uniquely informed by our companion systematic review and meta-analysis of preclinical studies in this area[215]. We summarise the key findings and elaborate further on some of their implications for the field.

A systematic review of laboratory animal studies that employed models/conditions associated with persistent pain and reported a pain-relevant outcome measure, identified 473 published reports of which 374 reported data that could be included in a meta-analysis. Data from 6479 rats and 6876 mice respectively were included, reflecting 864 and 677 experimental comparisons, respectively. This is a very large data set by preclinical standards. No studies in other species were found. Ninety-nine studies (~20 %) were excluded from the meta-analysis because the methods and/or results were not reported in sufficient detail to permit extractable data to be included in a meta-analysis.

Overall, the data support the hypothesis of cannabinoid-mediated analgesia. The overall effect size (Hedge's G Standardised Mean Difference, SMD) was 1.32 [$Q = 4101.26$, d.f. 1543, $p < 0.0001$, $I^2 = 61.58$ %] (Figure 3). The models used reflect a range of conventional and diverse inflammatory and neuropathy paradigms, particularly surgically-induced nerve injury for the latter. With regard to outcome measures, in common with other pre-clinical behavioral pain studies, limb withdrawal measures evoked by sensory stimuli were by far the most frequently reported outcome measure, with very few reports of complex behavioral assessments. Lack of consensus on predictive validity of such measures limits the degree to which the presence of pain, and thus pain relief from the intervention, can be inferred[190,202]. This contrasts with the clinical trial literature where patient reported pain intensity is the predominant metric. The formalin test also features strongly in the data; this model of

tonic inflammatory pain entails measuring several spontaneous or non-evoked nociceptive behaviors e.g. licking, lifting, flinching, rearing, and guarding to generate a 'combined or composite pain score' which thereby affords a greater degree of confidence regarding the impact of pain on the animal's behavior[1,69,224].

Small molecule CB_1 and CB_2 receptor agonists and non-selective cannabinoid receptor agonists (including THC) were the most frequently assessed interventions. FAAH inhibitors and PPAR-α agonists (in particular palmitoylethanolamide; PEA) were also frequently evaluated. In general, studies demonstrated antinociceptive efficacy, as measured predominantly by attenuation of injury/inflammation-associated hypersensitivity in evoked limb withdrawal (Figure 4).

The differences between the effect sizes of the different interventions may be inherent to the intervention, e.g., mechanism of action, route of administration and dosing regimens but are also likely to be influenced by other study design characteristics e.g. the model, choice of species, strain, sex and behavioral outcome measure.

In rodent inflammatory pain models (e.g. formalin, Complete Freund's Adjuvant [CFA], carrageenan and osteoarthritis), CB_1, CB_2 receptor agonists and PEA consistently attenuated pain-related behaviors across a range of inflammatory pain models (Table 3). The only exception was for carrageenan-induced inflammation in rats, for which CB_1 receptor agonists did not significantly attenuate pain-related behaviours. The efficacy of FAAH inhibitors was mixed; pain-related behaviors were significantly attenuated in formalin and CFA but not in osteoarthritis rodent models. In carrageenan models, a species difference was detected in which FAAH inhibitors significantly attenuated pain-related behaviors in mice but not rats. THC also significantly attenuated pain-related behaviors in formalin, CFA and carrageenan models. Like FAAH inhibitors, the efficacy of CBD was mixed; pain-related behaviors were significantly attenuated in formalin and CFA but not in carrageenan and osteoarthritis rodent models.

In rodent neuropathic pain models e.g. nerve injury, chemotherapy-induced peripheral neuropathy and diabetes, CB_1 and CB_2 receptor agonists, FAAH inhibitors and CBD consistently demonstrated antinociceptive efficacy (Table 4). PEA also significantly attenuated pain-related behaviors in nerve injury and chemotherapy models. THC significantly attenuated pain-related behaviors in nerve injury models.

The meta-analysis suggests that the most frequently assessed cannabinoids, CB_1 and CB_2 receptor agonists and non-selective agonists, consistently attenuated pain-associated behaviors in a broad range of inflammatory and neuropathic pain models. Although tested in fewer model types, this was similarly evident for THC. CBD and FAAH inhibitors were not as effective in inflammatory pain models but may be a viable candidate for the treatment of neuropathic pain. However, this analysis does not take into account potential side effects e.g. motor impairment, hypothermia or anxiolysis, that could influence the behavioral outcomes. Prospective preclinical trials are required to better ascertain what factors are influencing the differences in observed efficacy between the different drug classes and model types.

The systematic review highlights several differences between the preclinical and clinical assessment of candidate treatments. The number and profile of the drugs assessed preclinically (171 different interventions) differs markedly from those investigated in clinical trials (11 different interventions in[79]) where the predominantly evaluated clinical interventions are pharmacologically complex cannabis-based medical extracts, THC or THC analogues. Indeed, an unusual feature of the cannabinoid field is that the initiation of clinical trials has not generally followed (or has been very slow to follow) the publication of preclinical data providing evidence of benefit for small molecule drugs, whereas this is the case for many other drug classes. Moreover, certain indications assessed in clinical trials (e.g. pain due to third molar extraction) are not represented in the preclinical literature.

The routes of administration also differ, and accompanying pharmacokinetic investigation was only evident in 7% of the studies. However, 69 % of studies did confirm CB_1 or CB_2 receptor involvement through the use of antagonists, transgenic mice or radioligand binding.

The systematic review also highlights several weaknesses in face and construct validity of the animal models and there are several study characteristics that can impact pain-associated behavioral outcome measures and the assessment of novel antinociceptive efficacy. The animal cohorts are genetically very similar, converse to the widely heterogeneous patient population. In rats, most studies employed either Sprague-Dawley or Wistar strains, although larger effect sizes were evident in the small number of studies reported with Lewis rats. There was a major bias to the use of male rats (91%). Similar findings were found in mice with 81% of studies reporting the use of males across 29 strains. This does not reflect the clinical situation where women are overrepresented among patients with chronic pain [164]. In clinical trials male and female patients are more equally represented e.g. a recent systematic review of 36 randomised controlled trials assessing cannabinoids, cannabis, and CBMs, female patients outnumbered (n=3691) male patients (n=3613) [79].

More generally, the animal models do not effectively simulate multidimensional clinical pain conditions including the psychological component. Disease or injury is frequently induced in young, otherwise healthy animals contrasting with the clinical situation in which disease or injury predominantly occurs in older patients with co-morbidities. The duration of animal studies is usually brief (up to a few weeks) which results in candidate treatments being tested in the early stages of disease onset which does not adequately reproduce the impact of prolonged clinical pain nor address the clinical need for treatment in the later stages of disease. There is an over reliance on evoked limb withdrawal, a measure of hypersensitivity, which is not appropriate for pain that is characterized by sensory loss or spontaneous pain. Careful consideration should be given to the choice of species, strain, sex and age in relation to the clinical condition being modelled. To limit threats to external validity, researchers should balance the sexes[39]. A broader range of outcome measures that are of clinical relevance and include more complex, ethologically relevant behaviors is required[202]. Multicenter testing will increase environmental heterogeneity and study samples thereby improving the generalizability of preclinical findings[231].

As with the vast majority of current preclinical neuroscience and pharmacological research employing animal models, the risk of bias is uncertain, due to a generic poverty of reporting

sufficient details of the experimental design, conduct and analysis factors which govern the veracity of experimental internal validity. There is evidence to suggest that low prevalence of the reporting of measures to mitigate bias tend to give higher estimates of treatment effects[48,105]. The meta-analysis did not show a consistent relationship between the reporting of methodological quality criteria and smaller effect sizes, however, larger effect sizes were reported for studies that did not report allocation concealment and sample size calculations. In relation, the methods by which biases were mitigated were also infrequently reported. On the rare occasions where they were, the methods were often invalid[230] e.g. randomization by 'picking animals randomly from a cage' rather than computer generated random sequence, or determining sample size based upon reported sample size norms rather than a power calculation. Thus, there is a critical need for transparency of reporting all experimental details so that the quality of research can be assessed, and the validity of the outcomes inferred.

There was also evidence of publication bias overestimating the effect size reported in the literature because of the well-known propensity to report studies which support the hypothesis, and historic difficulty of publishing data that show no effect. It is also to be expected that animal studies will yield larger effect sizes in comparison to clinical studies due to the more homogenous nature of the study population, and better opportunity to control experimental variables, reducing the observed variance and making direct comparison of effect sizes impossible.

Our accompanying systematic review and meta-analysis therefore highlights the need for improvements in experimental design and, perhaps even more importantly, the reporting of experimental design and analysis features in sufficient detail such that primary research can be reproduced and meta-analysed. Nevertheless, notwithstanding the caveats relating to animal studies of analgesia, substantial evidence from animal model experiments supports the hypothesis of cannabinoid-induced analgesia in inflammatory and neuropathy conditions.

Side effects relevant to preclinical pain studies

An important consideration in the evaluation of any pain medication is whether analgesic efficacy is accompanied by adverse side effects that limit therapeutic potential, or additional beneficial effects that might enhance therapeutic potential. The present evaluation of on-target "side effects" is restricted to effects of cannabinoids/endocannabinoid system modulators administered to rodents in adulthood. Additional preclinical research is needed to specifically assess potential harms in vulnerable populations (e.g. older adults, and the developing fetal and adolescent brain[40,41,91]). In this section, we consider both potential harms and benefits of pharmacological strategies assessed *specifically* in animal pain models, particularly as they may emerge with chronic dosing. We also consider pharmacological effects (e.g. motor impairment, hypothermia or anxiolysis) that could influence interpretation of pain-related behavior. Animal models have been used to investigate an array of mechanisms and sites of analgesic action using a much broader array of mechanistically distinct compounds compared to those evaluated in the clinical literature. Most therapeutic interventions that show promise in preclinical studies, nonetheless, fail

in clinical trials, albeit for different reasons (i.e. efficacy, side effect profile, lack of adequate target engagement, therapeutic indication, variability in clinical populations, clinical primary outcome measure); this failure rate is not unique to cannabinoids. Moreover, animal models may not adequately capture adverse side effects that may be problematic in humans, and adverse side effects are often not systematically studied in animal experiments. Nevertheless, preclinical studies have provided evidence of both beneficial (i.e. antinociception, anti-emetic, anti-spasticity, anti-stress, anti-anxiety-like effects) and adverse (i.e. reward or aversion, dependence, tolerance, motor and memory impairment) effects of cannabinoids and endocannabinoid system modulators in otherwise normal animals (for review see[183,184]). Here, we primarily consider "on-target" pharmacological effects of cannabinoids in pain models only, including assessments of cannabimimetic effects (i.e. in the classic cannabinoid tetrad), tolerance, physical dependence, reward/reinforcement, opioid sparing effects, antinociceptive synergy and effects on stress-, anxiety- and depression-related behavior in animal models of pain.

Cardinal signs of CB_1 receptor activation.

The classic cannabinoid tetrad assesses cardinal signs of CB_1 activation (i.e. hypoactivity, reduction in body temperature, catalepsy, and tail-flick antinociception)[156,214] in rodents and is produced by all CNS-penetrant CB_1 agonists, including THC. These cannabimimetic effects are consistent with localisation of CB_1 to motor and limbic regions in rodent brain[104]. Drug-induced motor impairment can mask detection of antinociception in rodents. Consequently, preclinical studies must show that antinociceptive effects of cannabinoids observed in behavioral studies are not artifacts of motor impairment. Whereas motor effects complicate behavioral evaluation of antinociceptive effects of cannabinoids, they do not preclude the existence of antinociceptive mechanisms. Our accompanying systematic review revealed that a minority (33%) of studies assessed the effects of the drugs on motor activity[215]. Direct CB_1 agonists and non-selective cannabinoid receptor agonists that penetrate the CNS have potential to produce undesirable CB_1-mediated pharmacological effects in humans (e.g. psychoactivity, motor and memory impairment) (for review see[183,184]). Electrophysiological measures of activity of nociceptive neurons and/or neurochemical measures of noxious stimulus-evoked neuronal activation provide independent lines of evidence that non-selective cannabinoid receptor agonists, CB_1 agonists, FAAH inhibitors, and CB2 agonists suppress nociceptive processing in rodents (for review see[98,99,217,240]). Non-selective cannabinoid receptor agonists (e.g. WIN55,212-2) suppress electrophysiological[112,114,115,158] and neurochemical markers of pain-evoked neuronal activation[226] as well as pain behavior at doses that do not alter body temperature or produce immobility[186,199]. Drug-induced reductions in body temperature cannot explain electrophysiological or behavioral indicators of antinociceptive efficacy[158,170,228]. WIN55,212-2-induced reduction of evoked hypersensitivity in the chronic constriction injury (CCI) model of neuropathic pain, and side effects (motor incoordination, catalepsy and sedation), can occur at similar ED50s in mice[3], however, antinociceptive effects of WIN55,212-2 correlate with suppression of firing in nociceptive neurons and is reported to outlast these motor effects[158].

Drug development efforts have focused on elucidating therapeutic potential of small molecules that engage targets within the endocannabinoid system that lack unwanted cannabimimetic effects associated with direct CB_1 activation (for review see[50,113,139]). CB_2 agonists, FAAH inhibitors, MGL inhibitors (at appropriate doses), CB_1 positive allosteric modulators (PAMs), peripherally-restricted non-selective cannabinoid receptor agonists (PrNM1; [166]) have all been shown to suppress pain behavior without unwanted motor effects of CB_1 agonists (for review see[98,99,217,240]). High dose MGL inhibitors can elicit tetrad effects whereas a dual FAAH/MGL inhibitor JZL195 suppressed neuropathic nociception in the mouse CCI model with an ED50 four times lower than that which produced side effects (motor incoordination, catalepsy, sedation)[3], although brain permeant and impermeant FAAH inhibitors lack such effects[5,73,197,223]. Dual FAAH/MGL inhibitors represent a pharmacological strategy to elevate both AEA and 2-AG and produce antinociceptive efficacy without producing unwanted cannabimimetic effects in the tetrad[66]. CB_1 knock-out (KO) mice have also been used to assess the impact of CB_2 activation without the confound of CB_1-mediated side-effects[56,66,126,208]. Such studies show that mixed cannabinoid agonists and CB_2 agonists can engage CB_2 receptors independently of CB_1 to alleviate neuropathic pain-related behavior induced by paclitaxel administration or spinal nerve liagtion[56,126].

The abundance of CB_1 in brain regions controlling motor activity and memory accounts for adverse side effects of mixed and CB_1-preferring strategies[104]. Very few studies evaluate possible memory impairment induced by pharmacological treatments in laboratory animal pain models. However, studies that involve learning approaches document that rodents in pathological pain states can show conditioned place preferences[50,84] or perform operant responses[102,166] to chambers/tasks associated with pain relief; memory impairment would preclude demonstrations of efficacy in such studies. Peripherally-restricted CB_1 agonists, CB_2 agonists, MGL inhibitors and CBD represent cannabinoid modulators that have shown efficacy in such assays in animal pain models[50,84,166].

Tolerance

Tolerance, the loss of therapeutic efficacy with repeated administration, is undesirable in an analgesic and can lead to dose escalation and potential for misuse and abuse. In animal models of pathological pain, tolerance to therapeutic efficacy develops to direct acting CB_1 agonists, non-selective cannabinoid receptor agonists, as well as high, but not low, doses of MGL inhibitors[56,142,212], presumably via downregulation and desensitization of CB_1 receptors. In a mouse model of chemotherapy-induced neuropathic pain induced by paclitaxel, tolerance developed to the antinociceptive effects of THC and other non-selective cannabinoid receptor agonists (CP55,940, WIN55.212-2), as well as to other classical cannabimimetic effects[56,212]. Low dose chronic infusion of WIN55,212-2 and AM1241 (CB_2 agonist) were associated with sustained antinociceptive efficacy in the paclitaxel model without motor impairment[199]. Tolerance develops more quickly to high compared to low doses of the centrally acting CB_1-preferring ligands[56,212]. By contrast, antinociceptive tolerance is typically absent in neuropathic as well as inflammatory pain models following repeated administration of CB_2 agonists[56,199,212], brain permeant and impermeant FAAH inhibitors[38,212,213], low dose (but not high dose) MGL inhibitors[142],

CB1 PAMs[128,212] and a peripherally restricted CB_1 agonist[166]. The FAAH/MGL dual inhibitor JZL195 exhibited greater efficacy than FAAH or MGL inhibitor alone and did not produce tolerance, exhibiting an improved therapeutic window compared to direct cannabinoid CB_1 agonists[3]. Further preclinical studies are necessary to determine whether different therapeutic strategies (i.e. biased CB_1 agonism, peripherally restricted non-selective cannabinoid receptor agonists) can separate therapeutic efficacy from unwanted side effects.

Physical dependence

Receptor antagonists have been used to precipitate a withdrawal syndrome, a sign of physical dependence, in mice subjected to pathological pain states. In mice rendered neuropathic with paclitaxel, the CB_1 antagonist rimonabant precipitates signs of physical dependence (i.e. paw tremors) in mice treated chronically with orthosteric cannabinoid agonists (i.e. THC, CP55940, WIN55,212-2); severity of withdrawal symptoms was dose-related[56,212]. In the same neuropathic pain model, both tolerance and rimonabant-precipitated withdrawal signs were produced by the MGL inhibitor JZL184 but not by a CB_2 agonist[56,212], brain permeant or impermeant inhibitors of FAAH[213] or a CB_1 PAM[212]. Where present, physical dependence was induced by challenge with CB_1, but not CB_2, antagonists and in animals receiving direct acting agonists (or high dose MGL inhibitor) that penetrated the CNS. By contrast, neither CB_1 PAMs nor brain permeant or impermeant inhibitors of FAAH were associated with signs of physical dependence following 3 weeks of once daily administration in paclitaxel-treated mice[212,213]. Notably, CB_1 antagonist-precipitated withdrawal syndromes (i.e. paw tremors) lacked the more striking somatic (i.e. jumping) and autonomic (i.e. diarrhea) signs associated with naloxone-precipitated opioid withdrawal[9,212].

Reward/Reinforcement

THC can produce both rewarding and aversive effects in laboratory animals[35,151,216]. Rewarding properties of cannabinoids, have, historically, been demonstrated in otherwise healthy, young, male rodents which may not necessarily mimic the situation in people with chronic pain. In pathological pain states, interpretation of positive reinforcing effects of cannabinoids (i.e. reward in the typical drug abuse sense) should be differentiated from negative reinforcing effects (i.e. removal of an aversive pain state). Preclinical research involving rodent pain models has investigated therapeutic strategies targeting the endocannabinoid system that hold promise for suppressing pain without producing abuse liability. Using a classic drug self-administration approach, rats rendered neuropathic by a spared nerve injury self-administered a CB_2 agonist in a CB_2-dependent manner; naïve animals did not reliably self-administer the drug[102]. Moreover, CB_2 agonists did not produce conditioned place preference (CPP)[93,130]. These observations suggest that the CB_2 agonists were not inherently reinforcing in the *absence* of the pathological pain state. In the absence of pathological pain, CB_1 PAMs ZCZ011 and GAT211 do not produce CPP when administered alone[129,212]. Moreover, ZCZ011 did not substitute for CB_1 agonists CP55940 or AEA in a drug discrimination assay [129] suggesting it, does not produce cannabimimetic side effects. Inhibition of MGL with MINI110 also reversed a negative affective state associated with paclitaxel treatment using a CPP approach[50]. Similarly, a non-rewarding dose of CBD produced reward-related effects in the presence of pain due to incisional

injury[84]. Thus, in the absence of pathological pain state, CB_1 PAMs, and endocannabinoid deactivation inhibitors did not produce rewarding or aversive effects when administered alone in rodents. These studies raise the possibility that components of the endocannabinoid signaling system may be targeted for therapeutic benefit without the rewarding properties of direct CB_1 activation in the CNS.

Opioid sparing effects and antinociceptive synergy

Opioids remain a mainstay of pain management but also produce tolerance, physical dependence, reward, constipation, and respiratory depression, among other effects. Efficacy of adjunctive therapies for suppressing pathological pain and producing opioid sparing effects has, consequently, been evaluated. THC produced synergistic antinociceptive effects with morphine in both arthritic (Freund's adjuvant-induced) and naïve rats[45]; additive, rather than synergistic, interactions were reported for unwanted side effects (e.g. tetrad, motor ataxia)[45]. WIN55,212-2 produced synergistic antinociceptive interactions with morphine in mice with CCI of the sciatic nerve but only additive effects on motor coordination[3]. Brain permeant and impermeant inhibitors of FAAH[212], MGL inhibitors[237], dual FAAH-MGL inhibitor[66], CB_2 agonists[93,130] and CB_1 PAMs[211] produce synergistic antinociceptive effects with morphine in neuropathic pain models. The CB_2 agonist JWH133 produced additive antinociceptive effects with morphine in the formalin test[245]. Notably, in a mouse neuropathic pain model, brain permeant (URB597) and impermeant (URB937) FAAH inhibitors[213], a CB_1 PAM (GAT211;[211]) and CB_2 agonists (AM1710, LY2828360;[9,153,212]) suppressed development of morphine tolerance without enhancing naloxone-precipitated opioid withdrawal. Multiple CB_2 agonists attenuated opioid tolerance and naloxone-precipitated opioid withdrawal in neuropathic mice[9,130,153,155,212]. CB_2 agonists produce synergistic antinociceptive effects with opioids and also attenuate opioid-induced respiratory depression[236,246]. Neither FAAH inhibitors (URB597, URB937) nor a CB_1 PAM (GAT211) enhanced naloxone-precipitated opioid withdrawal in paclitaxel-treated mice[212,213]. Evaluations of other opioid side effects in the same studies (i.e. slowing of GI motility, reward), have typically employed normal animals not subjected to pathological pain states. Nonetheless, lowering opioid doses required to elicit therapeutic effects could enhance therapeutic ratios. Most studies have combined opioids with endocannabinoid modulators that themselves lack observable cannabimimetic side effects (JWH015, MJN110, URB597, URB937); consequently, additivity of adverse side effects would not be expected. Interestingly, CB_2 agonists (JWH015; LY2828360) produced synergistic anti-allodynic effects with morphine in models of inflammatory (formalin), post-operative (paw incision) and neuropathic (SNI) nociception but did not produce synergy for nociceptive pain[93,130]. Synergy between CBD and morphine has also been reported in the acetic acid-induced writhing model[176] and coadministration of THC and morphine reduced the second phase of formalin-evoked nociceptive behaviour in rats to a greater extent than either drug alone[78]. More work is necessary to examine whether cannabinoids alter other unwanted side effects of opioids (e.g. slowing of GI motility) in pain models, and evaluate the clinical relevance of these findings

Synergism of antinociceptive efficacy: Non-opioid analgesics

THC has been shown to produce synergistic antinociceptive interactions with gabapentin in the mouse CCI model of neuropathic pain[9]. Synergy between CBD and THC, and between PEA and gabapentin is also reported in chemotherapy-induced neuropathic pain models[66,67,141], between CBD and THC in the mouse CCI model of neuropathic pain [34], and between PEA and acetaminophen (paracetamol) in the rat streptozotocin (STZ)-induced model of diabetic neuropathy[53]. The COX-2 inhibitor celecoxib increased the antihypersensitivity activity of the CB_1 agonist Met-F-AEA and the CB_2 agonist AM1241 in the STZ model[26]. Low doses of the MGL inhibitor JZL184 and the non-selective COX inhibitor diclofenac synergistically attenuated mechanical allodynia and additively reduced cold allodynia in the mouse CCI model of neuropathic pain[49]. Furthermore, co-administration of the FAAH inhibitor URB597 and diclofenac yielded synergistic antinociceptive effects in the acetic acid-induced abdominal stretching model of visceral nociception in mice[174]. In other work, anandamide co-administered with either ibuprofen (non-selective COX inhibitor) or rofecoxib (selective COX-2 inhibitor) resulted in synergistic antinociceptive effects in the rat formalin test[97,101]. Mechanistically, it is important to note that there are a number of interactions between COX/NSAIDs/acetaminophen and cannabinoids/endocannabinoid system, including inhibitory effects of NSAIDs or an acetaminophen metabolite on endocannabinoid catabolism or transport (for review see[189]). Thus, adjunctive therapies could enhance therapeutic ratios of existing treatments for both inflammatory and neuropathic pain. CB_1 PAMs enhance antinociceptive and unwanted side effects of orthosteric CB_1 agonists[128,212] and produce synergistic anti-allodynic effects with FAAH and MGL inhibitors without enhancing other tetrad parameters[212].

Beneficial on-target pharmacological effects

Beneficial on-target pharmacological effects (i.e. in suppressing stress, anxiety, nausea and producing improvements in sleep) of cannabinoids (for review see[183,184]) could contribute to perceived therapeutic benefits in relevant pathological pain states. However, only small numbers of studies have evaluated such features specifically in animal pain models. Indeed, our accompanying systematic review revealed that only 3% of studies assessed the anxiolytic or anti-depressant-like effects of the drugs in the animal models of injury-related or pathological persistent pain[215]. Systematic preclinical studies are necessary to determine whether, rather than purely modulating sensory thresholds, cannabinoid-based modulation of affective behavior could contribute to therapeutic efficacy of analgesics by attenuating symptoms that exacerbate pain.

Stress, anxiety, depression and pain-depressed behavior

The therapeutic potential of cannabinoids/endocannabinoid system modulators has been assessed in animal models of anxiety, stress, and depression (for review see[76,90,165,183,184,187]). However, fewer studies have evaluated such therapeutic effects within the context of pathological pain states (for review see[42,80,217,240]). The CB_2 agonist GW405833 reduced immobility (i.e. a measure of depression-like behavior) in the forced swim test and allodynia (although mediation by CB_2 was not assessed), whereas

the anti-depressant desipramine primarily attenuated immobility time[120]. In the mouse monosodium iodoacetate (MIA) model of osteoarthritis pain, the CB_1 agonist ACEA and the CB_2 agonist JWH133 ameliorated nociceptive and affective alterations, and ACEA also improved associated memory impairment[147]. In rats with CCI, the brain permeant FAAH inhibitor URB597 but not the brain impermeant FAAH inhibitor URB937 suppressed immobility in the forced swim test, and attenuated novelty-induced suppression of feeding and CCI-induced reductions in hippocampal neurogenesis, all indicative of anti-depressant-like effects, whereas both URB597 and URB937 suppressed allodynia[132]. Thus, the CNS penetrant FAAH inhibitor produced beneficial effects on both evoked pain and pain-induced depression-like behavior. Rats with spared nerve injury exhibited an anxiety-like phenotype relative to shams; repeated CBD prevented anxiety-like behavior in the open field test, elevated plus maze and novelty-induced suppression of feeding test, in addition to suppressing allodynia via a $5\text{-}HT_{1A}$ receptor mechanism[52]. Thus, effects of CBD revealed in that study are likely to be independent of cannabinoid receptor activation.

In addition to evaluations of responses to evoked pain (pain-stimulated behaviors), an emerging preclinical literature has evaluated pain-depressed behavior (e.g. marble burying, nestlet shredding, intracranial self-stimulation thresholds). While the extent to which assays of pain-depressed behavior assess stress-, anxiety-, or depression-related behavior is uncertain, they represent an attempt to more completely model the multifaceted complexities associated with pathological pain states[177]. Assessing the effects of drugs on pain-depressed behavior may also help to distinguish between antinociception and motoric side-effects of putative analgesics. THC and CP55,940 produced antinociception for pain-stimulated responding in an acid-induced writhing assay but exacerbated pain-depressed behavior (i.e. noxious stimulus-induced suppression of intracranial self-stimulation thresholds)[146,150]. By contrast, the FAAH inhibitor URB597 suppressed both pain-stimulated as well as pain-depressed behavior[145]. A MGL inhibitor (MJN110), CB_2 agonist (LEI101), non-selective cannabinoid receptor agonist (CP55940), and reference analgesics (morphine, gabapentin, valdecoxib) all reversed pain-stimulated behavior (i.e. mechanical hypersensitivity) as well as pain-depressed behavior (i.e. deficits in marble burying) in mice with CCI[50]. By contrast, the benzodiazepine diazepam reversed neither dependent measure whereas a kappa-opioid receptor agonist (U69593) and a FAAH inhibitor (PF3845) reversed deficits in mechanical hypersensitivity only[50]. Deficits in marble burying resolved within a week of surgery[50] and not all neuropathic pain models are associated with deficits in marble burying/nestlet shredding[212]. Thus, the translational relevance of these behaviors to pain and/or anxiety, consequently, remain unclear but may they provide insights of some relevance to general health/quality of life.

Sleep

Effects of cannabinoids on sleep have not been assessed in animal pain models. Clinical data on the FAAH inhibitor PF-04457845 in cannabis use disorder have suggested improvements in both withdrawal and sleep[51]. More work is necessary to ascertain presence of sleep disruptions in laboratory animal models of pathological pain states and ascertain whether endocannabinoid modulators could restore such deficits.

Pharmacokinetics of cannabinoids

In addition to the mechanisms of drug action that affect the endocannabinoid system, and cannabinoid structure-activity relationships (i.e. pharmacodynamics), the assessment of drug pharmacokinetics is of critical importance both for better understanding the preclinical pharmacology, and for improving animal-to-human translation[235]. The pharmacokinetic processes of drug absorption to the systemic circulation, its distribution to tissues of interest, as well as its metabolism and excretion will determine how much drug, after a particular dose delivered via a particular route, will reach its site of action. In addition, drug pharmacokinetics will determine the duration of a drug's effect, and additional parameters related to drug-drug interactions and dose adjustments required in cases when organs such as liver or kidneys do not function properly.

Animal models provide advantages for investigating drug pharmacology because endogenous systems often cannot be manipulated safely in humans. However, since drug pharmacokinetics can be investigated directly in humans, this section of the manuscript will also discuss clinically-relevant pharmacokinetic data, where possible. Pharmacokinetic properties of cannabinoids, particularly their absorption, demonstrate substantial variability as a function of both route of administration, and the specific formulation in which the cannabinoids are delivered. Human studies repeatedly demonstrate large inter-subject variability in the pharmacokinetics of identical cannabinoid products, the basis of which is currently not well understood. Therefore, comparisons of non-human and human pharmacokinetics can have important translational relevance.

Absorption

Cannabinoid absorption is generally faster via the inhalational route than oral route, resulting in a more rapid onset of pharmacological effects, and shorter time to peak effect. In addition, cannabinoid delivery via the inhalational route circumvents the variability in oral absorption processes that are due to first pass metabolism. The limitations of inhalational routes include the variability in inter-individual efficiency that is caused by differences in inhalation techniques, respiratory tract irritation during inhalation, and the inconvenience or lack of adherence associated with smoked, vaporised, or nebulised cannabinoid products. While most preclinical experiments are performed with injected or oral/gastric administration of cannabinoids, most clinical therapeutic studies have used either inhaled products, oromucosal products such as nabiximols/Sativex®, or oral THC and its analogues.

The reported oral bioavailability of cannabinoids, and THC in particular, varies as a function of drug vehicle, and the co-ingested food. For example, orally ingested cannabis-containing cookies demonstrate only 6% THC bioavailability, while THC dissolved in sesame oil delivered in soft gelatin capsules is ~20% bioavailable (for review see[122]). A similar pattern of improved oral bioavailability approaching 30%, when dissolved in sesame oil, has been reported in rat studies with both THC and CBD[131].

The bioavailability of THC delivered via smoked cannabis products reportedly varies between 18 to 50%[180]. In a small study comparing absorption of a physiologically

compatible, nebulised inhalation solution of THC with that of intravenous THC in eight healthy volunteers, the average bioavailability of THC delivered via the inhalational route was approximately 28%[172], somewhat comparable to average bioavailability with smoking. However, the measured area under the THC concentration time curve (AUC) among study participants varied more than 5-fold after intravenous administration, and more than 15-fold after inhalation. Adverse effects, as expected, were more prominent with intravenous dosing[172].

After oral THC administration, the time to reach maximum plasma concentration (Tmax) has been reported between 30 and 120 min (but up to 6 hours in some studies) and is comparable between young and older adults[4]. Similarly, a wide-range of Tmax values, between 1-8 hours, has been reported with rat studies of THC, depending on the fasted/fed status, and the formulation in which THC is administered[131,198]. However, peak plasma concentrations (Cmax) of THC appear to be highly variable, with 4-7 fold differences among individuals, particularly in older adults[4]. With single oral doses of THC, the average Cmax ranged between 1.5-3 ng/mL after 3-6.5 mg doses[4], and approximately 7ng/mL after 20mg oral THC (dronabinol) administration[171]. With inhaled doses, 0.053 mg/kg nebulised THC (mean dose 3.5mg)[172] resulted in average Cmax of 20ng/mL,and with smoked cannabis, THC doses of 0.25 mg/kg body weight (14-22mg per cigarette) have resulted in mean Cmax of 48ng/mL. The plasma concentration profiles of key THC metabolites, namely 11-COOH-THC-, and 11-OH-THC, also vary substantially among people. With such variability in absorption and plasma concentrations of some cannabinoids, it is not surprising that clinical trial results are inconsistent[79].

CBD, considered by many as devoid of the psychotropic side effects of other cannabinoids, has garnered attention in recent years. After oral administration, CBD follows close-to-linear absorption (e.g. Cmax of 530 ng/mL with 3000mg/day, and 780ng/mL with 6000mg/day oral dosing), but high-fat meals can increase CBD plasma exposure and peak plasma concentrations by 4-5 fold[82,221]. Following doses of 10 mg CBD with 10 mg THC either in an oral capsule or oromucosal nabiximols spray, mean reported Cmax was 2.5-3 ng/mL for CBD and 6.1-6.4 ng/mL for THC[138].

Cannabinoids are highly lipophilic molecules with low aqueous solubility, and are susceptible to degradation and oxidation, especially in a solution. Drug formulation can thus play a crucial role in increasing the solubility and physicochemical stability of cannabinoid drugs, thus improving their pharmacokinetic properties[25]. Commonly used strategies in marketed products include pH adjustment, use of co-solvents, the use of micelles and nanoemulsions, complexation with cyclodextrins, or encapsulation in lipid-based formulations such as liposomes and nanoparticles. Even simple approaches such as administration of THC and CBD in lipid-rich oral formulations can increase plasma bioavailability of cannabinoids 2-3 fold, compared to lipid-free formulations[248].

Formulations that are based on self-(nano)emulsifying drug delivery technology (SEDDS) have been proposed as a means of improving the oral bioavailability of drugs that show poor aqueous solubility, including cannabinoids. This approach results in a consistent increase

in oral bioavailability of both THC (9-fold) and CBD (6-fold) in rats[37], and a 1.5-fold and 2.2-fold increase respectively, in humans[36].

Despite structural similarities, oral bioavailability differs between different cannabinoids. Nabilone, a synthetic cannabinoid derivative, is only slightly structurally different from THC (and dronabinol); however, it has substantially higher bioavailability than dronabinol (95% vs. 10–20%). One proposed difference relates to relative affinity to chylomicrons, and therefore the extent of lymphatic absorption of a particular cannabinoid[85]. Drug formulations containing long-chain triglycerides (LCT) may substantially improve the lymphatic absorption of selected cannabinoids[85]. Cannabinoids have demonstrated immunomodulatory effects *in-vitro*; however, plasma concentrations achieved with clinically-relevant dosing are well below those shown to affect lymphocyte function. By choosing the appropriate lipid-based formulation, lymphatic absorption of orally administered cannabinoids can be improved to achieve 100-fold and 250-fold higher lymph (vs plasma) concentrations of THC and CBD, respectively, which could help in the targeting of conditions where neuro-immunological effects of cannabinoids are of interest[247,248].

While there is high variability with the oral routes, consideration of variability related to smoked or vaporised delivery is also important. In pulmonary delivery routes, the depth of inhalation, how long breath is held for, and vaporiser temperature all affect cannabinoid absorption[25]. Inhalational devices that are temperature-regulated to control the inhaled doses may allow less variable pharmacokinetic profile of inhaled cannabinoids[70].

The transdermal route of administration provides an alternative approach for systemic delivery of lipophilic compounds with highly variable oral absorption, but more research is required to optimise these delivery systems and assess their efficacy and safety profiles[188,229].

Distribution

Most naturally occurring cannabinoid receptor ligands are lipophilic, and readily penetrate the blood-brain barrier, and permeate well to lipid-rich tissues such as the brain and peripheral fat. Cannabinoids display slower elimination rates from these tissues compared to plasma[24]. THC achieves higher concentration in rodent brain than in plasma, and CBD achieves approximately a 1:1 ratio[106]. Many of the synthetic ligands behave similarly. For example, WIN 55,212-2 and MK-9470, cannabinoid receptor ligands commonly used in preclinical studies, demonstrate similarly high brain:plasma penetration ratio in rodents[163,194].

In rhesus monkeys exposed to either intermittent (days) or long-term (years) cannabis consumption, the pharmacokinetics of single dose subcutaneous THC were not substantially different[87]. However, physiological responses of change in core temperature, or behavioral response time were different, and less profound, in monkeys with chronic exposure, likely suggesting physiological/behavioral tolerance to THC. Although blood THC levels were highest at 30-min post-injection, maximal effects on temperature and response rate did not occur until 120-min after injection, after blood THC levels had substantially

decreased, suggesting a non-linear relationship between blood concentration of THC and its pharmacodynamic effects.

The existence of peripheral antinociceptive mechanisms[32,81]for review see[107,152,169,196,204,240,250] prompted development of peripherally-restricted cannabinoid receptor ligands. However, developing such compounds that are not lipophilic but have high binding affinity to CB receptors has been challenging. Several compounds meeting the desired criteria of limited brain penetration with high affinity to CB_1 and CB_2 receptors have been synthesized[2]. Some achieve 0.16-0.18 brain:plasma ratio and produce antinociceptive effects in a rat model of neuropathy without producing catalepsy, but are yet to be evaluated clinically. Brain impermeant inhibitors of endocannabinoid metabolism have also been developed and show promise in preclinical studies of inflammatory and neuropathic pain[38,100,212,213].

There may be sex-specific differences in cannabinoid pharmacokinetics. Plasma levels of THC after intraperitoneal injection are comparable between male and female rats but levels of THC metabolites in brain tissue, including 11-OH-THC, the major active metabolite, were higher in female than male rats. When behavioral effects such as response to heat nociception and catalepsy were tested, SKF525A, a cytochrome P450 inhibitor, attenuated THC-induced antinociception and catalepsy in female, but not in male rats. Greater levels of active THC metabolites produced by females could potentially contribute to greater behavioral effects of THC in female compared to male rats[225]. Human studies have found somewhat similar tendencies, with 11-OH-THC levels higher in female after THC administration, along with higher subjective ratings of drug effects[210].

Metabolism and Excretion

Cannabinoids undergo a variety of metabolic processes in the gut, the liver, and various other tissues. Many metabolites have been identified, but the relative activity and toxicity of each is unknown. Once absorbed, THC is primarily oxidized by the cytochrome P450 hepatic mixed-function oxidase system to equipotent 11-hydroxy-THC (11-OH-THC), and further metabolized to inactive 11-COOH-THC. Most studies assessing the metabolic profile of THC have measured the plasma concentrations of these two metabolites, 11-OH-THC and 11-COOH-THC. The area under the concentration-time curve (AUC) of the psychoactive metabolite 11-OH-THC is 4-6 times lower compared to the AUC of THC itself after inhalational or IV administration, but overall, 11-OH-THC has a similar pharmacokinetic profile and elimination half-life[171,172].

11-COOH-THC, the main cannabinoid metabolite detected upon urine drug screens, has average terminal elimination half-life of 3-5 days. However, due to high inter-patient variability, both in adults and adolescents, 11-COOH-THC can be detected in plasma or urine a month or more after biochemically-verified abstinence[209].

CBD undergoes multiphasic elimination. Its effective half-life estimates ranged from 10 to 17 hours in humans, and the terminal elimination half-life is approximately 2-3 days[221]. The effective half-life of some of its metabolites, including 7-carboxy-cannabidiol (COOH-CBD), is around 24 hours, with plasma concentrations in hundreds of ng/mL detectable

many days after single dose administration. In rats and mice, the half-life of CBD has been reported in the range of 1-4 hours, with some unexplained variability among administration by different routes and in different formulations[20,54,137,244].

When oral CBD (Epidiolex®) was administered in people with variable extent of liver impairment, the total exposure (AUC) to CBD was increased by 50% in subjects with mild hepatic impairment, 2.5-fold in subjects with moderate, and 5-fold in severe hepatic impairment[221].

There is debate in the literature on whether CBD potentiates or antagonises analgesic effects produced by THC. One hypothesis is that CBD is an inhibitor of CYP450 enzyme systems, and may affect the pharmacokinetic profile of THC. CBD does inhibit microsomal CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 enzymes, but appears to do so at much higher concentrations than typically achieved in plasma with clinically-relevant doses[138]. In addition, in a human study of oromucosal THC and CBD administration, no major differences in THC pharmacokinetics were observed in the presence or absence of co-administered CBD[138].

In summary, cannabinoids demonstrate substantial inter-individual variability in pharmacokinetics, particularly in absorption processes, whether via oral or inhaled routes. This can result in differences in drug concentrations at the desired sites of action, leading to inconsistent clinical effects. Only a minority of preclinical studies of cannabinoids and endocannabinoid system modulators in animal pain models have assessed drug pharmacokinetics. Particular attention is required in the future studies to understand the pharmacokinetic-pharmacodynamic relationships of drugs that interact with the endocannabinoid system, to improve translational success.

Discussion, conclusions and future perspectives

Preclinical laboratory animal models provide an opportunity to investigate molecular and cellular changes associated with cannabinoid-based interventions. Pharmacological, electrophysiological, genetic and optogenetic methodologies can facilitate understanding of the involvement of the endocannabinoid system in neural circuits, and the opportunities to modulate the system to interfere with nociception and pain behaviour.

Key differences exist between preclinical and clinical evaluations of cannabinoids, CBMs and endocannabinoid system modulators. Substantial evidence from the preclinical literature supports the hypothesis of cannabinoid-induced analgesia at multiple levels of analysis[215], while there is less evidence of efficacy in human patients with pain and evidence is identified to be of low or very low-quality[79]. Foremost amongst these differences is that the clinical and preclinical literatures generally evaluate different compounds. For example, preclinical animal studies rarely test cannabis itself, while most human studies use whole cannabis or cannabis extracts containing THC and CBD in various ratios or doses and a limited number of other cannabinoids (e.g. THC or synthetic THC). Indeed, very few preclinical animal studies have evaluated vaporised/inhaled THC; none of the preclinical studies included in our meta-analysis assessed the effects of THC or cannabis administered

in vaporised/inhaled form, and only 38 preclinical studies have administered cannabis extracts via other routes[215]. A recent preclinical brain imaging study showed that inhaled vaporised cannabis plant enriched in THC (10.3% THC; 0.,05% CBD) uncoupled brain resting state functional connectivity in the raphe nuclei in paclitaxel-treated rats, normalizing paclitaxel-induced hyperconnectivity to levels observed in vehicle-treated rats, and also produced antinociception in the cold plate test[7]. The paucity of such studies may reflect difficulty in administering cannabis plant material to rats and mice in a manner where the dose of THC and other phytocannabinoids administered would be precise. Preclinical animal studies have performed mechanistic evaluations using individual cannabinoids and endocannabinoid system modulators (or sometimes combinations of two or more of these), at very well-defined doses and via routes of administration (mostly parenteral) that yield predictable pharmacokinetics.

Second, a much wider variety of cannabinoids and endocannabinoid system modulators have been tested in animal models of pain than have been assessed in human pain patients. This point is underscored by comparing our IASP Task Force clinical and preclinical systemic reviews, which reviewed 11[79] and 171[215] pharmacological interventions, respectively. Ethical, safety and methodological standpoints require that novel/experimental drugs are administered in rodent models before human subjects, and where mechanism of action can also be identified. Significant barriers to performing clinical research with cannabis and cannabinoids also remain. There are, collectively, over 150 preclinical studies assessing the efficacy of CB_2 receptor agonists, endocannabinoid re-uptake inhibitors, MGL inhibitors and FAAH inhibitors in animal models of pain, but not a single randomised controlled clinical trial (RCT) of the first 3 drug classes, and only 3 RCTs assessing the efficacy of FAAH inhibitors in pain patients[19,123,232]. This phenomenon, wherein the clinical studies lag significantly behind the preclinical studies, is, of course, to be expected, and is certainly not unique to the cannabinoid field. However, cannabis and cannabinoid use in humans has bypassed the usual preclinical and clinical studies typical of conventional drug development because of the widespread illicit, or more recently licit, use of cannabis by the public. The disparity that exists between the variety of cannabinoids and endocannabinoid system modulators that has been tested in animal models of pain versus what has been assessed in human pain patients should be kept in mind when comparing or contrasting efficacy of specific cannabinoids in animal models versus cannabis in human patients (in addition to translational relevance of the animal model and appropriateness of the clinical indication).

Another key difference between preclinical and clinical studies is that the latter very often administer the cannabinoid or cannabis as adjunctive/add-on treatment, alongside other analgesics that the patient is using. In contrast, preclinical studies in animals almost always investigate the cannabinoid drug against a ‘clean’ background, in the absence of any other analgesics (unless drug-drug interactions are specifically investigated). When administered adjunctively, the ‘window’ or opportunity to see therapeutic efficacy of the cannabinoid/cannabis may well be smaller than if it were investigated against a ‘clean’ background; if the other analgesics are already having partial effects in lowering pain, it may be more difficult for an add-on cannabinoid/cannabis to induce a further measurable reduction superimposed upon the pre-existing reduction.

Our accompanying systematic review and meta-analysis provides a comprehensive summary of studies in which cannabinoids, cannabis-based medicines and endocannabinoid system modulators were assessed for anti-nociceptive efficacy in animal models of injury-related or pathological persistent pain[215]. The overall behavioural data effect sizes are significant and it is clear that there is a spectrum of efficacy dependent on drug and pain model type, and also on species, strain and other experimental factors.

The risk of internal and external validity related biases confounding these data and exaggerating effect sizes is uncertain, but not insignificant. This could be better ascertained if future pre-clinical studies were reported with sufficient transparency to ascertain the methods that were used to mitigate against such biases[8,121,140,143,201,203]. There is a need for preclinical living systematic reviews that incorporate new evidence as it becomes available[71], and closer, multi-disciplinary, cross sector collaboration to ensure that laboratory animal studies have sufficient rigor to identify promising candidate drugs and more accurately inform clinical trial design.

Several intervention strategies offer the potential for analgesia while also circumventing adverse side effects of direct acting CB_1 agonists. These include cannabinoid CB_2 agonists, peripherally restricted CB_1 agonists, inhibitors of endocannabinoid deactivation (especially FAAH inhibitors), synergism between cannabinoids and other analgesics, mode of drug delivery (e.g. local, targeted delivery), biased agonism, CB_1 PAMs and CBD. FAAH represents a promising target whose engagement is likely to show a reduced pattern of unwanted CB_1-mediated effects, produce anxiolytic effects and opioid-sparing effects without being inherently reinforcing. Moreover, the recent case report of a woman with a mutation in the FAAH gene who does not experience pain or anxiety provides further support that FAAH inhibitors may not only exhibit reduced adverse side effects compared to conventional analgesic but may also exhibit additional desirable therapeutic properties (i.e. reduction of stress, fear and anxiety)[64,160]. With regards to reduction of abuse liability, CB_2 receptor agonists deserve especial mention for their potential to produce anti-addiction effects[9,17,130,175,212,249]. CBD represents another promising therapeutic agent which does not produce THC-like effects in humans or animals[95,127,173]. The mechanism underlying therapeutic effects of CBD remains incompletely understood[52,95]. Preclinical studies nonetheless, suggest that CBD may attenuate opioid addiction[124,125], THC withdrawal[168], reduce nausea[154,185], suppress inflammation and nociceptive behavior[9,17,43,195,243] and attenuate stress responding[14,16,33,47,72,119,136,149]. Further preclinical studies of underlying mechanisms as well as clinical trials of CBD are warranted to better ascertain the therapeutic effects that may be exploited as adjunct or unitary therapy. There is also a pressing requirement to better understand the pharmacology and therapeutic potential of the other phytocannabinoids in the cannabis plant beyond THC and CBD, and whether/how these may interact with THC and CBD in the context of analgesia. Given the substantial inter-individual differences in cannabinoid pharmacokinetics among humans, future research is needed to improve the consistency of drug delivery methods, for better translational of preclinical developments to human studies. Preclinical laboratory animal models are evolving to capture aspects associated with pathological pain states observed clinically e.g. spontaneous pain, anxiety, stress and depression comorbidities, conditions under which dynamic changes in endocannabinoid tone, enzyme activity or receptors may be observed

(for review see[42,76,80,90,165,187]). Consequently, future preclinical studies in laboratory animals, that evaluate novel cannabinoid interventions, reference analgesics, as well as drug development candidates that previously failed for efficacy, are required to validate treatments, understand mechanisms of action, and enhance clinical translation.

Acknowledgements.

This work is part of the effort of the IASP Presidential Taskforce on cannabis and cannabinoid analgesia. We would like to thank Dr. Emer Power and Darragh Mattimoe for assistance with preparation of figures, and Dr. Christopher Eccleston and Dr. Emma Fisher for comments on the manuscript.

Funding:

The International Association for the Study of Pain commissioned this work in the form of a Presidential Task Force and funded attendance for the authors at a working meeting in Washington DC, November 2019.

Conflicts of Interest

David P. Finn – Dr. Finn reports an Industry-Academia research grant from Alkermes Inc. and Science Foundation Ireland outside of the submitted work. He also reports research grants in the area of cannabinoids or the endocannabinoid system from Shionogi Ltd (Shionogi Science Programme), from B. Braun Ltd jointly with Science Foundation Ireland, and from the Irish Research Council, CNPq Brazil and EU INTERREG Programmes.

Simon Haroutounian – Dr. Haroutounian reports grants from Disarm Therapeutics, and advisory board/consultancy fees from Vertex Pharmaceuticals, Medoc Ltd and Rafa laboratories, outside the submitted work.

Andrea G Hohmann – Dr. Hohmann reports grants from the National Institutes of Health (National Institute on Drug Abuse, National Cancer Institute, National Institute of Neurological Diseases and Stroke), Indiana Addiction Grand Challenge and is a co-inventor on a provisional patent related to CB2-opioid interactions.

Elliot Krane – Dr. Krane has nothing to declare.

Nadia Soliman – Ms. Soliman has nothing to declare.

Andrew SC Rice – Dr. Rice is a Council Member of IASP and Chair of the Presidential Task Force of the IASP, He undertook consultancy and advisory board work for Imperial College Consultants-in the last 24 months this has included personally remunerated work outside of the submitted work for: Abide, Confo, Vertex, Pharmanovo, Lateral, Novartis, Mundipharma, Orion, Asahi Kasei, Toray & Theranexis. He was the owner of share options in Spinifex Pharmaceuticals from which personal benefit accrued between 2015 and 2019 upon the acquisition of Spinifex by Novartis. Prof Rice is a named inventor on the patents – Rice A.S.C, Vandevoorde S. and Lambert D. M. Methods using N-(2propenyl)hexadecanamide and related amides to relieve pain. WO2005/079771 pending, and Okuse K. et al Methods of treating pain by inhibition of vgf activity EP13702262.0/WO2013110945. During the conduct of the study Imperial College received grants funding to support Prof Rice's programme of research from Biotechnology and Biological Sciences Research Council (BBSRC), Medical Research Council (MRC), Wellcome Trust, Alan and Sheila Diamond Charitable Trust, British Pain Society, Royal British Legion and the European Commission (IMI2 (EQIPD); FP7 (Neuropain) and H2020 (Dolorisk)).

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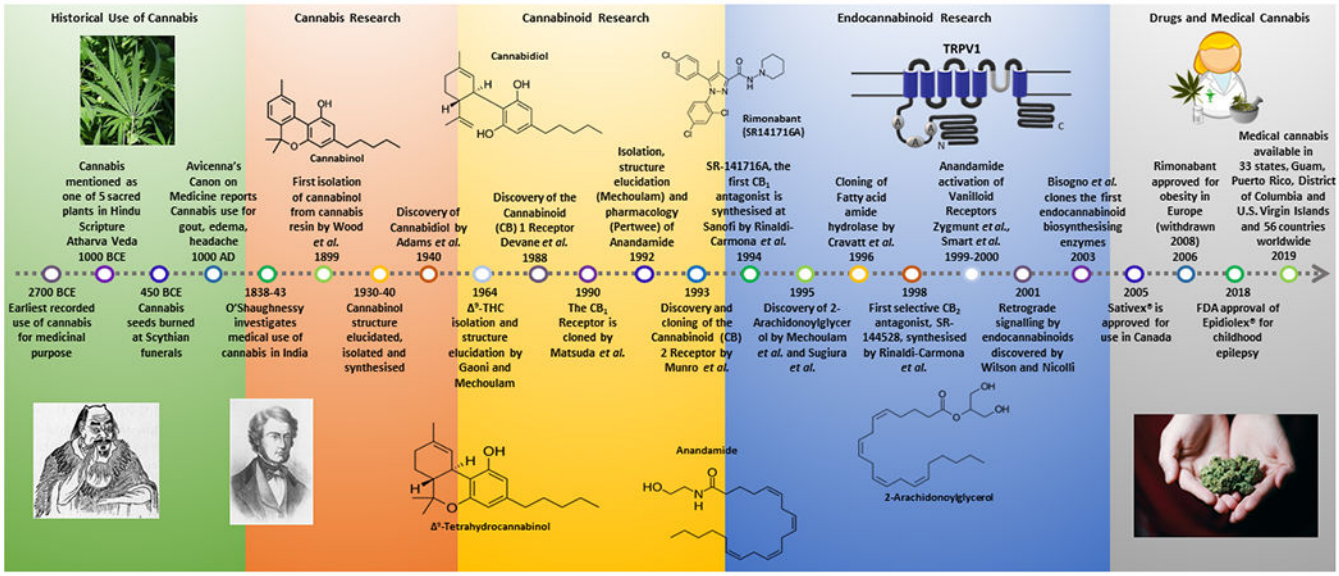

Figure 1.
A historical timeline of key milestones in cannabis and cannabinoid research.

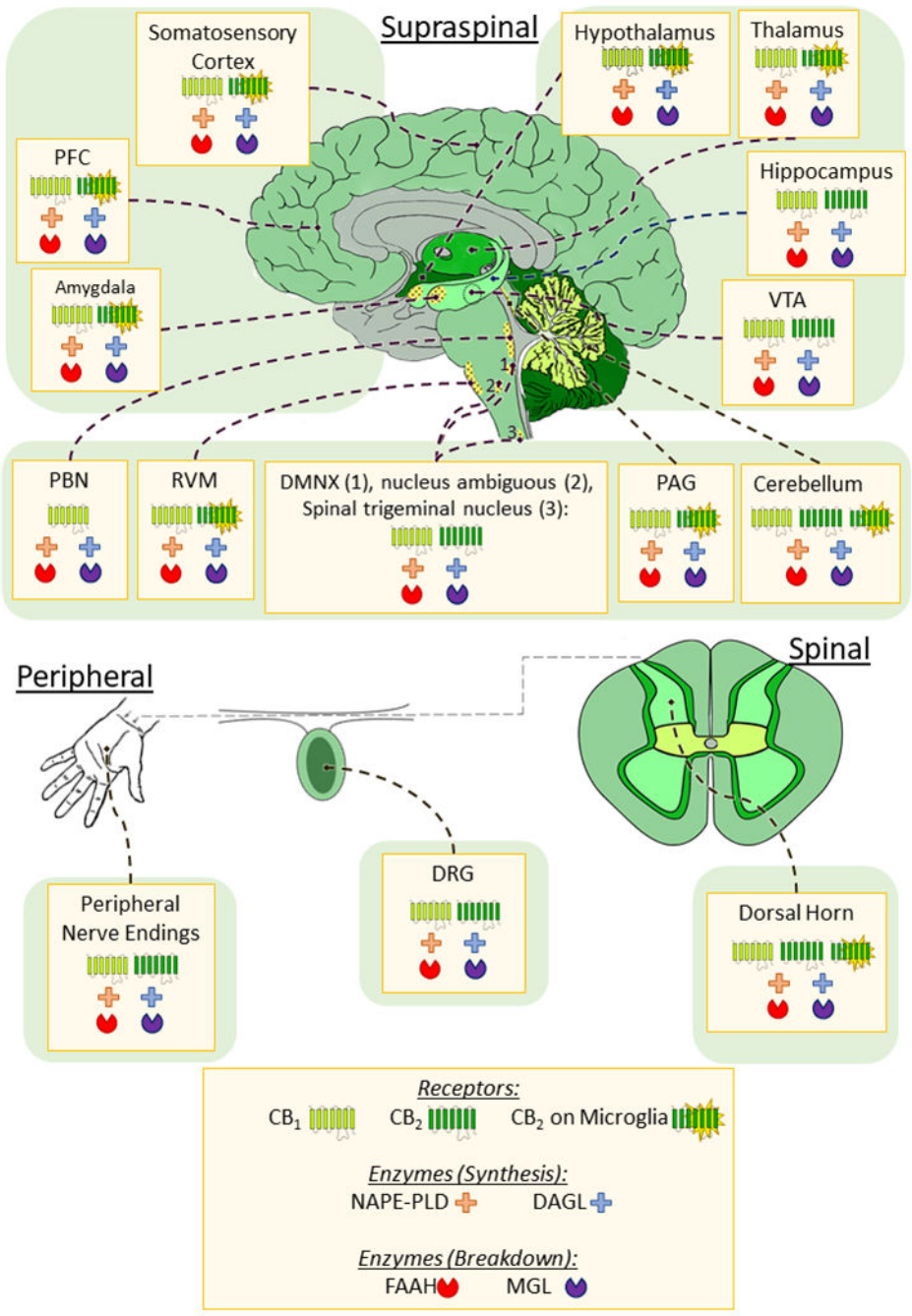

Figure 2.

Distribution of the cannabinoid receptors and enzymes associated with endocannabinoid synthesis and degradation in pain pathways. The endcannabinoid system is widely distributed throughout regions associated with pain processing and modulation in the brain, spinal cord and periphery; Most particularly the CB_1 receptor and the enzymes responsible for endocannabinoid synthesis (NAPE-PLD, DAGL) and degradation (FAAH, MGL). CB_2 receptors are less abundant in the brain and are primarily located on microglia, however studies have shown expression of CB_2 on neurons in the VTA, and several discrete nuclei

of the brainstem. CB_1 is also expressed on microglia at a lower level than CB_2, and effects of CB_1 on microglia may be mediated by neuronal CB_1. Therefore, this was not depicted above. In the DRG and periphery both CB_1 and CB_2 receptors can be found on neurons (and glia in the dorsal horn), as well as the endocannabinoid enzymes. PFC – prefrontal cortex; VTA – Ventral Tegmental Area; PAG – periaqueductal grey; RVM – rostral ventromedial medulla; PBN – parabrachial nucleus; DMNX - dorsal motor nucleus of the vagus nerve; DRG – dorsal root ganglion; CB – cannabinoid receptor; NAPE-PLD – N-acylphosphatidylethanolamine-hydrolyzing phospholipase D; DAGL – diacylglycerol lipase; FAAH – fatty acid amide hydrolase; MGL – monoacylglycerol lipase.

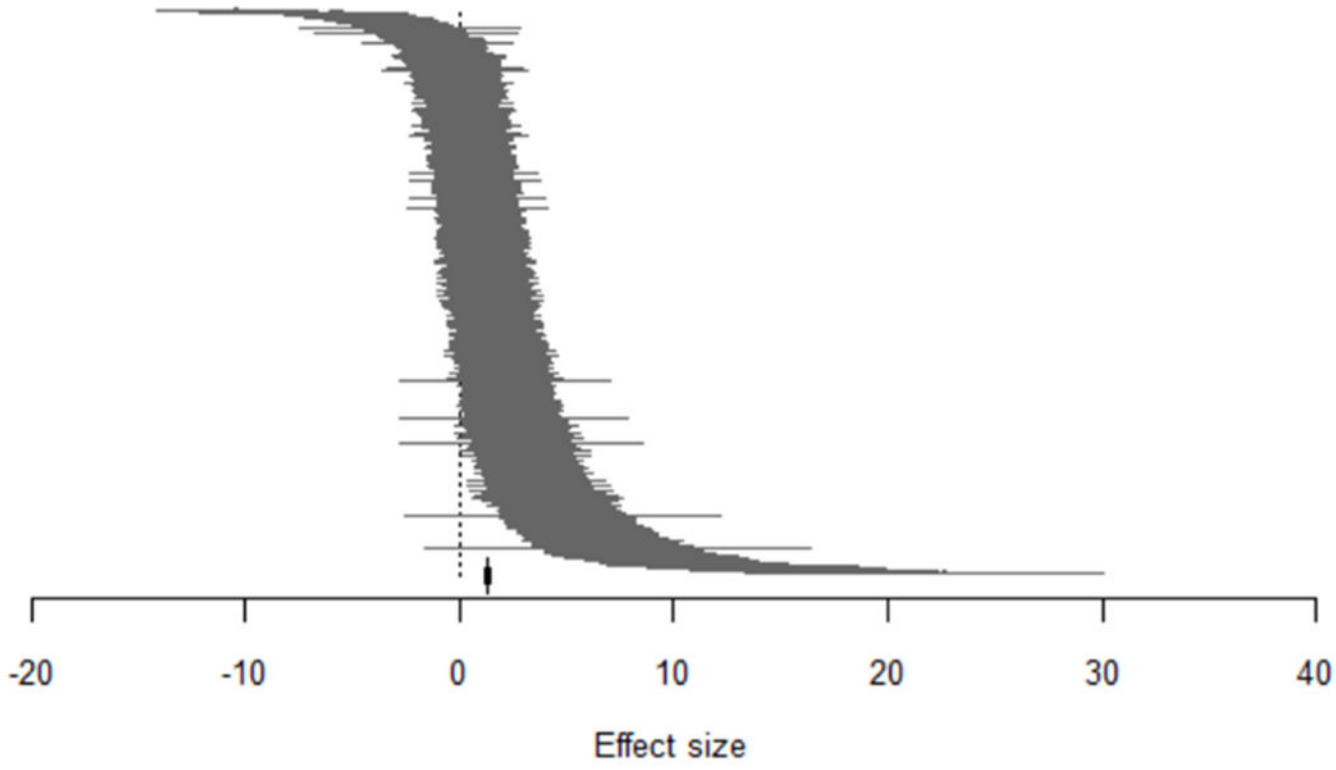

Figure 3.

The caterpillar plot of 1544 nested comparisons extracted from 374 studies included in the meta-analysis. A Hedge's G standardised mean difference effect size was calculated for each comparison. Overall effect size = 1.32[215]

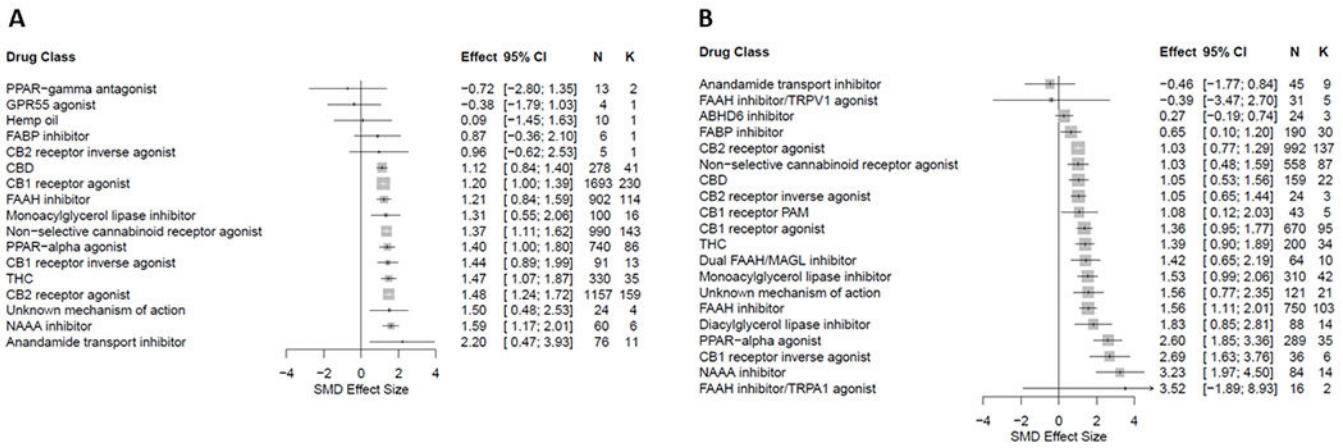

Figure 4.

Forest plot of drug classes assessed for antinociceptive efficacy in rat (A) and mouse (B) models of injury-related or pathological persistent pain. The size of the squares represents the weight (%) and its influence on the pooled result. N denotes the number of animals and K the number of comparisons of each sub-group[215].

Table 1.

Terminology and Definitions (Adapted from Soliman et al., 2019, after modification from Hauser et al. 2018).

Term	Definition	Examples/typical products
(Herbal) Cannabis	The whole plant or parts or material from the plant (e.g. flowers, buds, resin, leaves)	*Cannabis sativa*, hashish
Medical or medicinal cannabis	The term 'medical/medicinal cannabis' (or 'medical/medicinal marijuana') is used for cannabis plants, plant material, or full plant extracts used for medical purposes.	Bedrocan®, Bedrobinol®, Tilray 10THC/10CBD®
Cannabis-based (or cannabis-derived) medicines	Medicinal cannabis extracts with regulatory approval for marketing as a therapeutic with defined and standardized THC and/or CBD content.	Nabiximols (Sativex®), dronabinol (Marinol®), Epidiolex®
Cannabinoids	Cannabinoids are biologically active constituents of cannabis, or synthetic compounds, usually having affinity for and activity at cannabinoid receptors.	THC, CBD, CP55,940, WIN55,212-2, HU210, nabilone
Phytocannabinoid	A cannabinoid found in cannabis plants or purified/extracted from plant material	THC, CBD
Endocannabinoid	An endogenous ligand found in the body of humans and other animals and which has affinity for, and activity at, cannabinoid receptors	Anandamide, 2-AG
Modulators that decrease endocannabinoid system activity	Directly block cannabinoid receptors or reduce signalling indirectly via impeding action of endogenous ligand through actions at a distinct site	Cannabinoid receptor antagonists (rimonabant [SR141716A], AM251, SR144528, AM630), negative allosteric modulators (PSNCBAM-1), DAGL inhibitors (RHC80267)
Modulators that increase or enhance endocannabinoid system activity	In addition to individual phytocannabinoids, cannabis-derived or cannabis-based medicines, and cannabis extracts, other pharmacological approaches under development for manipulation of the endocannabinoid system include selective synthetic cannabinoid receptor agonists, inhibitors of the catabolism (e.g. fatty acid amide hydrolase [FAAH] inhibitors), transport (e.g. fatty acid binding protein [FABP] inhibitors) or reuptake of endocannabinoids, or positive allosteric modulators of cannabinoid receptor signalling.	FAAH inhibitors (PF-04457845, URB597, URB937), Anandamide transport inhibitors (AM404, VDM11), MGL inhibitors (URB602, JZL184, MJN110), Positive allosteric modulators of the CB_1 receptor (ZCZ011, GAT211)

CBD: cannabidiol; DAGL: Diacylglycerol lipase; FABP: fatty acid binding protein; THC: Δ^9-tetrahydrocannabinol; 2-AG: 2-arachidonoyl glycerol; MGL: monoacylglycerol lipase.

Table 2.

Cannabinoid Ligands/Preparations and Pharmacological Tools

Natural Cannabinoid Ligands/Synthetic Analogues	**CB_1-selective Agonists**
*Δ^9-THC (Dronabinol)	ACEA
*CBD	Met-F-AEA
* Cannabis extract, Δ^9-THC:CBD (2.5:1.25 mg)	AZ11713908 (peripherally restricted with CB_2 inverse agonist properties)
	Mixed CB_1/CB_2 Agonists
*Cannabis	BAY59-3074
*eCBD	CP55,940
*Nabilone (Δ^9-THC analogue)	*CT-3 (Ajulemic Acid)
*Nabiximols (oral-mucosal spray, Δ^9-THC:CBD, 2.7:2.5 mg)	HU-210
Endocannabinoids	WIN55,212-2
AEA	**CB_2-selective Agonists**
2-AG	AM1241
Endocannabinoid Modulators	AM1714
CB1 Positive Allosteric Modulators	AM1710
GAT211	A-796260
GAT229	A-836339
ZCZ011	*GW842166X
Uptake Inhibitors	*HU308
AM404	JWH015
LY2318912	JWH133
VDM11	LY2828360
OMDM132	MDA7
UCM-707	MDA19
FAAH Inhibitors	**CB_1 Antagonists**
OL135	AM251
*PF-00457845	AM281
URB597	AM6545 (peripherally restricted)
URB937 (peripherally restricted)	SR141716
MGL Inhibitors	**CB_2 Antagonists**
JZL184	AM630
URB602 (local only)	SR144528
MJN110	**Fatty Acids that do not bind CBRs**
Dual FAAH-MGL Inhibitors	NaGly
JZL195	PEA
SA57	L-29
FABP5 Inhibitors	
SBF126	

Abbreviations: AEA, anandamide; 2-AG, 2-arachydonoylglycerol; CBD, cannabidiol; eCBD, high CBD cannabis; Δ^9-THC, Δ^9-tetrahydrocannabinol; FAAH, fatty-acid amide hydrolase; FABP, fatty-acid binding protein; FLAT, FAAH-like anandamide transporter; MGL, monoacylglycerol lipase; N-arachidonoyl glycine, NaGly; PEA, palmitoylethanolamine;

*Denotes compounds used clinically; Adapted and updated from Hohmann and Rice (2013) Textbook of Pain 6th Edition and Rahn and Hohmann (2009) Neurotherapeutics 6: 713-3

Table 3.

Summary of the effects of cannabinoids and endocannabinoid system modulators on pain-related behaviour in the most frequently used rodent models of inflammatory pain. Adapted from[215].

	Formalin				Complete Freund's Adjuvant				Carrageenan				Osteoarthritis			
Drug Class	**K**	**N**	**Effect size**	**95 % CI**	**K**	**N**	**Effect size**	**95 % CI**	**K**	**N**	**Effect size**	**95 % CI**	**K**	**N**	**Effect size**	**95 % CI**
Anandamide transport inhibitor	10	64	1.97	0.608 - 3.342	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CB_1 receptor agonist	59	496	1.12	0.731 - 1.502	21	144	0.74	0.378 - 1.107	ns	ns	ns	ns	3	16	1.22	0.378 - 1.107
CB_1 receptor inverse agonist	1	7	1.71	0.427 - 3.002	NT	NT	NT	NT	5	30	0.44	0.055 - 0.822	NT	NT	NT	NT
CB_1 receptor PAM	NT	NT	NT	NT	NT	NT	NT	NT	1	8	2.45	1.067 - 3.837	NT	NT	NT	NT
CB_2 receptor agonist	33	265	0.81	0.316 - 1.307	44	338	1.10	0.829 - 1.366	10	74	1.39	0.885 - 1.896	24	173	1.42	0.829 - 1.366
CB_2 receptor inverse agonist	3	22	1.05	0.653 - 1.444	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
CBD	2	16	1.86	1.548 - 2.169	5	20	1.59	0.71 - 2.469	ns	ns	ns	ns	ns	ns	ns	ns
Dual FAAH/MAGL inhibitor	NT	NT	NT	NT	ns	ns	ns	ns	4	36	1.42	0.25 - 2.6	NT	NT	NT	NT
FAAH inhibitor	23	184	1.50	1.134 - 1.862	24	144	1.24	0.702 - 1.781	ns	ns	ns	ns	ns	ns	ns	ns
FABP inhibitor	ns	ns	ns	ns	15	98	0.78	0.194 - 1.373	6	36	1.42	0.947 - 1.895	NT	NT	NT	NT
Monoacylglycerol lipase inhibitor	8	69	2.28	1.382 - 3.175	ns	ns	ns	ns	8	60	2.67	0.967 - 4.378	ns	ns	ns	ns
NAAA inhibitor	NT	NT	NT	NT	3	30	1.63	1.235 - 2.025	12	72	1.91	0.923 - 2.897	NT	NT	NT	NT
Non-selective cannabinoid receptor agonist	38	253	1.56	1.019 - 2.11	ns	ns	ns	ns	45	311	0.74	0.202 - 1.268	ns	ns	ns	ns
PPAR-alpha agonist	21	143	1.73	0.701 - 2.761	2	16	2.53	0.851 - 4.205	26	173	3.26	1.51 - 5.006	9	75	0.98	0.851 - 4.205
THC	20	118	1.16	0.679 - 1.643	16	109	2.34	1.687 - 2.986	5	37	2.13	0.138 - 4.12	NT	NT	NT	NT

K denotes the number of comparisons and N denotes the number of animals within each sub-group. All entries with an effect size value were statistically significant. NT, not tested, ns, not significant.

Table 4.

Summary of the effects of cannabinoids and endocannabinoid system modulators on pain-related behaviour in the most frequently used rodent models of neuropathic pain. Adapted from[215].

	Nerve injury				Chemotherapy				Diabetes			
Drug Class	**K**	**N**	**Effect size**	**95 % CI**	**K**	**N**	**Effect size**	**95 % CI**	**K**	**N**	**Effect size**	**95 % CI**
Anandamide transport inhibitor	1	5	7.95	3.26 - 12.643	NT	NT	NT	NT	NT	NT	NT	NT
CB_1 receptor agonist	102	756	1.17	0.842 - 1.5	31	173	1.47	1.007 - 1.937	30	183	1.74	1.234 - 2.248
CB_1 receptor inverse agonist	4	36	1.71	1.171 - 2.248	NT	NT	NT	NT	2	12	4.53	1.932 - 7.127
CB_2 receptor agonist	87	639	1.15	0.812 - 1.497	27	192	1.549995	1.117 - 1.983	10	88	1.29	0.772 - 1.818
CBD	9	72	1.86	1.307 - 2.406	10	76	1.31	0.594 - 2.021	7	49	1.08	0.257 - 1.896
Diacylglycerol lipase inhibitor	1	6	3.69	1.235 - 6.148	ns	ns	ns	ns	NT	NT	NT	NT
Dual FAAH/MAGL inhibitor	2	12	2.08	1.348 - 2.808	NT	NT	NT	NT	NT	NT	NT	NT
FAAH inhibitor	59	439	1.75	1.212 - 2.281	19	148	1.96	1.048 - 2.868	17	180	2.53	1.455 - 3.604
Monoacylglycerol lipase inhibitor	16	119	1.31	0.774 - 1.84	12	80	1.42	0.179 - 2.653	NT	NT	NT	NT
NAAA inhibitor	5	34	4.54	2.594 - 6.496	NT	NT	NT	NT	NT	NT	NT	NT
Non-selective cannabinoid receptor agonist	75	504	1.18	0.825 - 1.536	ns	ns	ns	ns	8	48	1.89	0.335 - 3.437
PPAR-alpha agonist	26	229	1.14	0.493 - 1.786	11	74	2.54	1.341 - 3.746	NT	NT	NT	NT
THC	9	86	1.26	0.838 - 1.677	ns	ns	ns	ns	NT	NT	NT	NT

K denotes the number of comparisons and N denotes the number of animals within each sub-group. All entries with an effect size value were statistically significant. NT, not tested, ns, not significant.