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Introduction

Endocannabinoids in nervous system health and disease: the big picture in a nutshell

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The psychoactive component of the cannabis resin and flowers, delta9-tetrahydrocannabinol (THC), was first isolated in 1964, and at least 70 other structurally related 'phytocannabinoid' compounds have since been identified. The serendipitous identification of a G-protein-coupled cannabinoid receptor at which THC is active in the brain heralded an explosion in cannabinoid research. Elements of the endocannabinoid system (ECS) comprise the cannabinoid receptors, a family of nascent lipid ligands, the 'endocannabinoids' and the machinery for their biosynthesis and metabolism. The function of the ECS is thus defined by modulation of these receptors, in particular, by two of the best-described ligands, 2-arachidonoyl glycerol and anandamide (arachidonylethanolamide). Research on the ECS has recently aroused enormous interest not only for the physiological functions, but also for the promising therapeutic potentials of drugs interfering with the activity of cannabinoid receptors. Many of the former relate to stress-recovery systems and to the maintenance of homeostatic balance. Among other functions, the ECS is involved in neuroprotection, modulation of nociception, regulation of motor activity, neurogenesis, synaptic plasticity and the control of certain phases of memory processing. In addition, the ECS acts to modulate the immune and inflammatory responses and to maintain a positive energy balance. This theme issue aims to provide the reader with an overview of ECS pharmacology, followed by discussions on the pivotal role of this system in the modulation of neurogenesis in the developing and adult organism, memory processes and synaptic plasticity, as well as in pathological pain and brain ageing. The volume will conclude with discussions that address the proposed therapeutic applications of targeting the ECS for the treatment of neurodegeneration, pain and mental illness.

Keywords: endocannabinoids; immune modulation; synaptic plasticity; development; neurodegeneration; pain

1. INTRODUCTION: WHY STUDY ENDOCANNABINOIDS?

The *Cannabis sativa* plant has been exploited for medicinal, agricultural, recreational and spiritual purposes in diverse cultures over thousands of years. *Cannabis* seeds were initially used for food (6000 BC) and textiles made from its hemp (4000 BC) in China [1]. The first recorded use of *Cannabis* as medicine in Chinese pharmacopoeia dates to 2727 BC; *Cannabis* is later mentioned (1200–800 BC) in the Hindu sacred text Atharvaveda (Science of Charms) as 'Sacred Grass', one of the five sacred plants of India [1]. The psychoactive component of the cannabis resin and flowers, delta9-tetrahydrocannabinol (THC), was first isolated in 1964, and at least 70 other structurally related 'phytocannabinoid' compounds have since been identified. The development of synthetic cannabimimetic drugs has aided in the pharmacological characterization of an *endogenous* system, which responds to THC. The serendipitous identification of a G-protein-coupled cannabinoid receptor at which this compound is active in the brain gave birth to an explosion in endocannabinoid research which continues to this day. The *endocannabinoid* system (ECS) in the brain primarily influences neuronal synaptic communication, and affects biological functions—including eating, anxiety, learning and memory, reproduction, metabolism, growth and development—via an array of actions throughout the nervous system. In spite of recent scientific findings, there is much that remains to be discovered as to where and when endocannabinoids function, and how endocannabinoid signalling may be targeted for therapeutic benefit.

What makes the nervous system an even more compelling subject of scientific inquiry is the recent success medical science has had in other areas of disease and

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disability. As cancer death rates and cardiovascular disease rates begin to drop, what remains—and what looms even larger as a cause of human disease and disability—are diseases of the nervous system. Involvement of the ECS in nervous system health and disease will be a topic of interest to developmental biologists, cellular and molecular neurobiologists and pharmacologists, as well as basic and clinical scientists studying mental illness.

These developments are timely, in that they hold much promise to further our understanding of the consequences of endocannabinoid tone modulation in aberrant nervous system development, cognition, anxiety, anti-nociception and brain ageing, which, in turn, may translate into more effective treatments for such conditions than are currently available. Moreover, endocannabinoids are released by cell stress and physiologically appear to be immuno-modulatory. Among other functions, intriguing new evidence suggests that they are also important in maintaining a positive energy balance by modulating, among others, mitochondrial activity, and that this can provide a contextual background as to the function of this system in health and disease. Upsetting the latter may be a factor in the development of metabolic syndrome, which can lead to complications such as diabetes and is a risk factor for dementia and Alzheimer disease. This theme issue is intended to present the reader with the latest discoveries on the mechanism of action and potential therapeutic applications of endocannabinoids and endocannabinoid-like molecules in the nervous system.

2. THE MANY FACETS OF ENDOCANNABINOIDS

Research on cannabinoid signalling has grown at a remarkable pace, and some 5000 of the more than 15 000 PubMed citations to June 2012 are linked to endocannabinoids. One only needs to read a review on the topic published in this journal back in 2001 [2]. It is obviously impractical to cover all aspects within the limited space of this issue. The editors have therefore selected a team of respected investigators to highlight a number of key subthemes which we believe will furnish the reader with a current window on this subject.

(a) *Evolution and comparative neurobiology and signalling*

An understanding of mechanisms of endocannabinoid signalling is necessary to fully appreciate a discussion of the phylogenetic distribution of molecular components of this signalling system across the animal kingdom—including analysis of endocannabinoids, enzymes involved in their biosynthesis (e.g. diacylglycerol lipases, DAGLs) and inactivation (e.g. fatty acid amide hydrolase, monoacylglycerol lipase), receptors that mediate physiological effects of endocannabinoids (e.g. CB1, CB2) and proteins that regulate cannabinoid receptor activity. This volume begins with a chapter by Elphick [3], who takes us on an evolutionary tour of endocannabinoid signalling in the animal kingdom. This forms the basis of a presentation on selected examples of research on the neurophysiological roles of endocannabinoid signalling in non-mammalian animals. He shows how research on non-mammalian model animals has provided fundamental insights on the roles of endocannabinoid signalling in the nervous system as well as highlighting differences in endocannabinoid signalling systems in different animal types. Examples of model organisms that will be included in this survey are the leech *Hirudo medicinalis*, the sea squirt *Ciona intestinalis*, the zebrafish *Danio rerio* and birds (chick and zebra finch). The article concludes with a forward look, highlighting areas of interest for future studies on endocannbinoid signalling in non-mammalian animals.

Following the discovery of cannabinoid CB1 and CB2 receptors [4,5], the two specific G-protein-coupled receptors (GPCRs) for THC, several endogenous ligands (the endocannabinoids) were identified, the best known being anandamide and 2-arachidonoylglycerol (2-AG)). Other minor lipid metabolites different from, but chemically similar to, anandamide and 2-AG have also been suggested to act as endocannabinoids. In contradistinction to most other GPCRs (opioid receptors perhaps being the only other exception), cannabinoid receptors thus appear to have more than one endogenous agonist. In 1999, it was proposed that anandamide might also activate other targets, and in particular the transient receptor potential of vanilloid type-1 (TRPV1) channels [6]. These channels can also be activated by another less abundant endocannabinoid, *N*-arachidonoyldopamine, but not by 2-AG. As Di Marzo & De Petrocellis [7] point out, the capability of anandamide and 2-AG to be biosynthesized and inactivated independently from each other, and to interact not only with cannabinoid receptors but also activate or inhibit several molecular targets ranging from GPCRs to ion channels and nuclear receptors, allows for a very high degree of differential flexibility of their actions and for fine-tuning homeostasis. These authors also raise the intriguing idea that in the absence of strictly selective cannabinoid receptor ligands, CB1 and CB2 receptors might be part of a broader lipid-based signalling system, also involving, for example, other endogenous bioactive congeners and analogues of anandamide, and hence, other molecular signal transducers. This possibility could explain why anandamide and 2-AG are also found in invertebrates, such as molluscs, *Hydra* and *Caenorhabditis elegans*, which do not express CB1 or CB2 orthologues [8]. Indeed, irrespective of its role in the ECS, 2-AG is part of an established metabolic pathway.

(b) *Nervous system development, synaptic plasticity, learning and memory*

Cellular diversity in the nervous system evolves from the concerted processes of cell proliferation, differentiation, migration, survival and synapse formation [9]. Neural adhesion and extracellular matrix molecules play key roles in axonal migration and guidance towards synapses. The regulation of these processes in a precise manner depends on a complex network of molecular determinants and intracellular signalling pathways that are in turn modulated by surrounding information from the neurogenic niche [10]. The extracellular signalling pathways that modulate acquisition of the diversity of developing neuronal populations and determine their proper integration remain poorly

understood. Given the widespread and strong expression of ECS molecules, it is not surprising that exposure of the developing and maturing nervous system to marijuana-derived cannabinoid impacts behavioural aspects in the control of emotions and cognitive responses. Given the implications of cannabinoid exposure in human neuropsychiatric disorders (see Puighermanal *et al.* [11]; Melis & Pistis [12]; Campos *et al.* [13]), investigations on the mechanism of action and neurobiological substrate underlying the developmental action of cannabinoids have gained traction. Endocannabinoids, acting via the CB1 receptor comprise, in effect, neurodevelopmental signalling cues which exert a regulatory role on the molecular and cellular mechanisms involved in brain development. Galve-Roperh and co-workers [14] pick up the story and review experimental evidence supporting a functional role of the ECS during cortical development, based on conditional genetic and pharmacological manipulation studies. The CB1 receptor emerges as a novel signalling platform that drives neuronal generation and specification, modulating final brain maturation and connectivity. The CB1 receptor, in concert with locally produced endocannabinoids, regulates neural progenitor proliferation, pyramidal specification and axonal navigation. Further, localized subcellular endocannabinoid production acts as an axonal growth cone signal that regulates interneuron morphogenesis. These findings shed further light on the consequences of prenatal cannabinoid exposure, and highlight a novel role for endocannabinoids as neurogenic instructive cues in cortical development. Conceivably, altered CB1 receptor-mediated signalling may be a factor in developmental disorders and epileptogenesis [9].

Characterization of the subcellular distribution of components of the ECS more than a decade ago subsequently gave birth to a conceptual framework in which endocannabinoids would act as retrograde transmitters [2]. We now know that endocannabinoid signalling is responsible for both depolarization-induced suppression of inhibition [15,16] and depolarization-induced suppression of excitation [17], identifying it as a powerful and widespread modulator of synaptic strength. In his paper, Cachope [18] analyses the short-term forms of plasticity induced by endocannabinoids that have been described in numerous areas and in different organisms, describing not only hippocampus and cerebellum as the scenario for endocannabinoid action, but also the ECS an ancient mechanism in evolutionary terms. The fact that endocannabinoids have been identified as a trigger of long-term plasticity processes has prompted interest in how this system may be implicated in lasting phenomena, such as learning and memory, as will be discussed again later. Collectively, these observations propose that the ECS is very malleable and can establish specializations that lead to a much wider array of final effects than the traditional short- and long-term depression of glutamatergic and γ-aminobutyric acid-mediated transmission. Not unexpectedly, a neuromodulatory system with such a wide distribution in the brain would be predicted to be altered in numerous diseases and abnormal conditions. Indeed, for many of those illnesses, distribution of the CB1 receptor or levels of endocannabinoids seem to play crucial roles in the very initial, even pre-symptomatic stages.

The ECS is found at the pre- and post-synaptic side of the nerve terminals in brain areas involved in learning and memory, such as the hippocampus, which modulates synaptic function [19]. Neuronal activity is a potent stimulus for endocannabinoid synthesis and release [20] from post-synaptic neurons; these lipid mediators traverse the synapse to bind presynaptic CB1 receptors, thereby suppressing neurotransmitter release at both excitatory and inhibitory synapses in a short- and long-term manner. Ozaita and co-workers [11] delve into the cellular and molecular mechanisms underlying memory modulation by the ECS and its role in hippocampal synaptic plasticity, considering the implication of the ECS, the neuroanatomical basis for the effects of cannabinoids, and the cellular and subcellular localization of CB1 receptors in cognition. The widespread anatomical localization of CB1 receptors in the brain may explain its involvement in multiple memory stages that might require different neural substrates. In this context, several recent intriguing reports suggest the presence of CB1 receptors in astrocytes [21] and mitochondria [22] where they can also participate in the control of cognitive processes. In the brain, cannabinoids and endocannabinoids modulate a number of intracellular signalling pathways, some critically involved in the deleterious effect of cannabinoids on learning and memory processes. The involvement of the mammalian target of the rapamycin pathway and extracellular signal-regulated kinases, together with their consequent regulation of cellular processes such as protein translation, seem to play a critical role in the amnesic-like effect of cannabinoids. As well, there is emerging evidence for endocannabinoid roles in various forms of learning and memory outside the hippocampus, e.g. fear conditioning extinction and the amygdale [23,24], and habit learning and the basal ganglia [25].

Diacylglycerol (DAG) is one of the most extensively studied second messengers in cells. It is generated by the hydrolysis of phosphatidylinositol 4,5-bisphosphate in response to the activation of surface receptors that include GPCRs and receptor tyrosine kinases [26]. In addition to its many cellular functions, DAG can also serve as a substrate for enzymes that generate alternative signalling lipids [27]. One pathway of particular interest invokes the DAGLs which hydrolyse DAG to form 2-AG—the most abundant ligand for CB1 and CB2 receptors. Doherty and co-authors [28] describe how DAGL-dependent endocannabinoid signalling participates in synaptic plasticity at many levels: it regulates axonal growth and guidance during development; it is required for the generation and migration of new neurons in the adult brain; and at mature synapses, 2-AG released from post-synaptic terminals acts back on pre-synaptic CB1 receptors to inhibit the secretion of both excitatory and inhibitory neurotransmitters throughout the nervous system. It is intriguing that the DAGLs have functions beyond the cannabinoid receptors, serving as 'hub' enzymes in pathways that generate and/or maintain signalling pools of arachidonic acid in the brain and other organs. They are also emerging as key enzymes with

regulatory roles in pathological processes, including driving inflammatory responses implicated in neurodegenerative disorders. Although we still know very little about the mechanisms that regulate DAGL activity, these investigators have used homology modelling against other α/β hydrolases and a detailed examination of published proteomic studies and other databases to identify a regulatory loop with a highly conserved signature motif, as well as phosphorylation and palmitoylation as post-translational mechanisms to regulate DAGL activity and function, in concert with other fundamental processes, such as dynamic endocytosis.

(c) *The dopaminergic system and (mis)behaviour*

Dopamine (DA) neurons of the substantia nigra pars compacta (SNpc) and the more medial ventral tegmental area are associated with different functions: the nigrostriatal system with motor function (which degenerates in Parkinson's disease) and the mesolimbic system with motivation and reward functions, being essential for the habit-forming effects of drugs of abuse and for motivated behaviours. The ECS plays a modulatory role on reward DA neurons [29], and CB1 receptors together with the ligands anandamide and 2-AG are present within the ventral tegmental area [30]. Today we know a great deal about the role played by the endocannabinoid family lipids in several facets of DA neuron physiopathology. In their article prepared for this issue, Melis & Pistis [12] review recent advances that have shed light on understanding the differential roles of endocannabinoids and their cognate molecules in regulation of the reward circuit, and discuss their anti-addicting properties, particularly with a focus on their potential engagement in the prevention of relapse. Further, the authors suggest that anandamide and 2-AG, being intimately connected with diverse metabolic and signalling pathways, might differently affect various functions of DA neurons through activation not only of surface receptors, but also of nuclear receptors [31]. It is now emerging how DA neurons can regulate their constituent biomolecules to compensate for changes in either internal functions or external conditions, in effect using these lipid molecules as metabolic and homeostatic signal detectors. Since dysfunctions of the DA system underlie diverse neuropsychiatric disorders, including schizophrenia and drug addiction, understanding ECS dysfunctions related to either physiological and/or behavioural features of individuals vulnerable to drug addiction—if indeed they do exist—could open up new possibilities to treat drug addiction. The role of the nigrostriatal system in reward should not be overlooked, however, as this system supports intracranial self-stimulation [32,33], with nigral neurons participating in reward error detection just as readily as ventral tegmental area neurons [34].

(d) *Neuroinflammation, pain and neurodegeneration*

Nociceptive pain results from the detection of intense or noxious stimuli by specialized high-threshold sensory neurons (nociceptors), a transfer of action potentials to the spinal cord, and onward transmission of the warning signal to the brain. In contrast, clinical pain such as pain after nerve injury (neuropathic pain) is characterized by hyperalgesia in the absence of a stimulus and reduced nociceptive thresholds so that normally innocuous stimuli produce pain. The mechanisms involved in the latter are complex and involve both peripheral and central phenomena. Central sensitization is also associated with the activation and/or recruitment of glial cells within the nervous system which modulate neuronal responses through the initiation of multiple signalling cascades. The activation of microglia and astrocytes in the dorsal horn of the spinal cord plays a critical role in the development of facilitated nociceptive responses and spinal hyper-excitability in chronic pain models [35]. Chronic pain and neuropathic pain are indications for which there is high unmet need in the clinic [36]. Furthermore, chronic neuropathic pain can persist for months, without the underlying cause being treatable or identifiable. Indeed, only one in four patients experience over 50 per cent pain relief [36]. No single target has been shown to be uniquely associated with the establishment of neuropathic pain. However, among the pharmacological strategies under investigation, endocannabinoids and endovanilloids (that is, endogenous agonists at TRPV1 channels), in addition to enzymes that regulate their metabolism, represent a promising therapeutic avenue to a successful pain treatment. In a timely fashion, Starowicz & Przewlocka [37] update the relationship between CB1 receptors and TRPV1 channels and their possible implications for neuropathic pain, taking into consideration endogenous spinal mechanisms of pain control by anandamide, and the current and emerging pharmacotherapeutic approaches that benefit from the pharmacological modulation of spinal endocannabinoid and/or endovanilloid systems under chronic pain conditions. Emerging data suggest that the potential therapeutic effects of endocannabinoids can be strengthened by modulating their endogenous tone, i.e. by preventing their metabolism by various enzymes, mainly including the fatty acid amide hydrolase, in animal models of neuropathic pain. However, it may also be that endocannabinoids, produced upon strong nociceptive stimulation, activate CB1 receptors on inhibitory dorsal horn neurons in order to reduce the synaptic release of γ-aminobutyric acid and glycine—thus rendering nociceptive neurons excitable by non-painful stimuli. Spinal endocannabinoids and CB1 receptors located on inhibitory dorsal horn interneurons might thus act as mediators of heterosynaptic pain sensitization and play a hitherto unexpected role in dorsal horn pain-controlling circuits.

Cannabinoid ligands acting through CB1 receptors exert well-established analgesic effects. Their side-effect profile, however, has driven the search for alternative cannabinoid-based approaches to analgesia. Dynamic changes in the ECS, which are associated with chronic pain states, provide new opportunities for cannabinoid-mediated modulation of nociceptive processing, a topic which Chapman and co-workers [38] pick-up on. Starting with an overview of current knowledge on the impact of chronic pain states on the key components of the

endocannabinoid receptor system, we learn of localized changes in receptor expression and levels of key metabolic enzymes which influence endocannabinoid local levels. Emerging evidence suggests that spinal CB2 receptors have a novel role in the modulation of nociceptive processing in models of neuropathic pain, as well as in models of cancer pain and arthritis, and recent studies provide compelling evidence that 2-AG may be a key molecular player in endogenous inhibition at nociceptive synapses. Moreover, some of the metabolites of anandamide and 2-AG generated by cyclooxygenase-2, lipoxygenases and cytochrome P450 are biologically active and can either exacerbate, or inhibit, nociceptive signalling [39]. The complexities of endocannabinoid metabolism by multiple pathways, and their dynamic regulation by pathological conditions, need to be kept in mind when investigating the effects of drugs which specifically target fatty acid amide hydrolase or monoacylglycerol lipase as a strategy to elevate levels of anandamide and 2-AG.

Glia have emerged as key contributory players in central nervous system (CNS) disorders from neuropathic pain and epilepsy to neurodegenerative diseases. Although glia are well known for having a number of housekeeping functions that are necessary for healthy neuronal communication, on strong activation they act as immunoresponsive cells and release a number of glial and neuronal signalling molecules. Importantly, glial cells also respond to pro-inflammatory signals released from cells of immune origin, and in this context mast cells are of particular relevance. These immune-related cells, while resident in the CNS, are able to cross a compromised blood-spinal cord and blood-brain barrier in cases of CNS pathology. Emerging evidence suggests the possibility of mast cell–glia communication and opens exciting new perspectives for designing therapies to target neuroinflammation by differentially modulating the activation of non-neuronal cells normally controlling neuronal sensitization—both peripherally and centrally. Here, Skaper & Facci [40] provide an overview of glia and mast cell biology, and then go on to discuss what is presently known about molecules involved in endogenous protective mechanisms that may be activated as a result of tissue damage or stimulation of inflammatory responses and nociceptive fibres. One interesting group of such molecules are the *N*-acylethanolamines (or fatty acid ethanolamides), a class of naturally occurring lipidic mediators chemically similar to anandamide in as much as they are composed of a fatty acid and ethanolamine. Apart from anandamide (*N*-arachidonoylethanolamide), the principal fatty acid ethanolamide family members are *N*-stearoylethanolamide, *N*-oleoylethanolamide, and *N*-palmitoylethanolamide (PEA, or *N*-(2-hydroxyethyl)hexadecanamide). Numerous observations suggest that a key role of PEA may be to maintain cellular homeostasis when faced with external stressors provoking, for example, inflammation: PEA is produced and hydrolysed by microglia; it inhibits mast cell activation; it increases in glutamate-treated neocortical neurons *ex vivo* and in the injured cortex; PEA levels increase in the spinal cord of spastic (but not non-spastic) mice suffering from chronic relapsing experimental allergic encephalomyelitis. However, one may envision pathological settings where PEA endogenous production is insufficient to control the ensuing inflammatory cascade. A number of studies have addressed this issue by applying PEA exogenously, where it proved efficacious in mast cell-mediated experimental models of acute and neurogenic inflammation. PEA is endowed with neuroprotective effects as well: in a model of spinal cord trauma; in a delayed post-glutamate paradigm of excitotoxic death; and against amyloid β-peptide-induced learning and memory impairment in mice and organotypic hippocampal slices challenged with amyloid β-peptide. In mechanistic terms, the above actions appear to be mediated by PEA acting as an endogenous ligand for the peroxisome proliferator-activated receptor alpha. The latter belongs to a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes.

Ageing is associated with a decline of cognitive performance in humans and animals. Although the histological structure of the brain is largely preserved, intracellular signalling mechanisms undergo changes associated with the ageing process. Increasing concentrations of toxic metabolic end products, misfolded macromolecules and non-functional organelles characterize the ageing brain and are thought to significantly contribute to cognitive decline [41]. The balance between the generation and clearance of toxic metabolic by-products and damaged macromolecules crucially influences the progression of ageing. Within this context, could the ECS modulate molecular and cellular processes influencing the ageing process? In this volume, Bilkei-Gorzo [42] proposes that endocannabinoids indeed influence neuronal activity, exerting neuroprotective effects and regulating glial responses via the body's homeostatic defence system. The ECS influences the amount of intracellular reactive oxygen species not only via an anti-oxidant buffering capacity, but also by influencing the removal of damaged macromolecules and by regulating mitochondrial activity (discussed further below). Most CB1 receptors do not reach the cell surface, but instead are localized intracellularly at the lysosomal and late endosomal levels [43]. Cannabinoids at physiological concentrations increase lysosomal stability and integrity [44], but high concentrations of THC actually increase lysosomal permeability via the CB1 receptor. An intriguing question is whether decreased CB1 receptor activity owing to genetic variation or epigenetic changes in humans influences lysosomal function and thus contributes to the development of neurodegenerative diseases. Further, CB1 receptors have been suggested to also be present on mitochondrial membranes and regulate mitochondrial activity [22]. On the cellular level, the ECS regulates the expression of brain-derived neurotrophic factor and neurogenesis. Endocannabinoids are able to suppress neuroinflammatory processes contributing to the progression of normal brain ageing and to the pathogenesis of neurodegenerative diseases. Animals lacking CB1 receptors exhibit early onset of learning deficits associated with age-related histological and molecular changes, which supports the hypothesized role of endocannabinoids against brain ageing. The task now

falls to future clinical studies for confirmation of these preclinical findings.

ECS modulation of mitochondrial function is explored in greater depth by Nunn *et al.* [45], who posit that understanding a potential ECS-mitochondrial connection may serve to further explain the function of the ECS, especially how it might control cell fate by several different pathways (e.g. calcium, nitric oxide, ceramide production, mTOR)—thereby helping to explain its actions in the CNS. In this scheme, the ECS can be viewed as a stress response system with four actions, depending on dose: proliferation, suppression and adaptation, apoptosis and potentially, necrosis—each of which could be explained by its actions on the mitochondrion. Dose-related control of mitochondrial function could therefore provide insight into its role in health and disease, why it might have its own pathology, and possibly, new therapeutic directions. This may be especially pertinent for neurons, whose highly differentiated and post-mitotic status renders them highly reliant on oxidative phosphorylation of glucose.

(e) *Harnessing the therapeutic potential of endocannabinoids*

Can knowledge of cannabinoid biology be harnessed for therapeutic benefit? Currently, three medicines that activate cannabinoid CB1/CB2 receptors are in the clinic: Cesamet (nabilone), Marinol (dronabinol; THC) and Sativex (THC with cannabidiol (CBD)). These can be prescribed for the amelioration of chemotherapy-induced nausea and vomiting (Cesamet and Marinol), stimulation of appetite (Marinol), and symptomatic relief of cancer pain and/or management of neuropathic pain and spasticity in adults with multiple sclerosis (Sativex) [46]. Pertwee [47] provides an up-to-date account of where the field stands and mentions several possible additional therapeutic targets for cannabinoid receptor agonists. These include other types of pain, epilepsy, anxiety, depression, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, stroke, cancer, drug dependence, glaucoma, autoimmune uveitis, osteoporosis, sepsis and hepatic, renal, intestinal and cardiovascular disorders. In his article, he also describes potential strategies for improving the efficacy and/or benefit-to-risk ratio of these agonists in the clinic. These involve: (i) targeting cannabinoid receptors located outside the blood-brain barrier; (ii) targeting cannabinoid receptors expressed by a particular tissue; (iii) targeting upregulated cannabinoid receptors; (iv) selectively targeting cannabinoid CB2 receptors; and/or (v) adjunctive 'multi-targeting'. Minimizing the adverse effects of cannabinoid receptor agonists in the clinic requires careful consideration of the likely strengths and weaknesses for improving the therapeutic efficacy and/or limiting the adverse effects. Indeed, for many their strengths outweigh identified weaknesses. As the bulk of this information derived mainly from preclinical research, there is a clear need for phase I clinical trials with healthy human subjects and phase II trials with patients. It will also be important to select the strategy that would be the one most likely to produce the greatest benefit-to-risk ratio in patients [47].

CBD is the main non-psychotropic phytocannabinoid present in the *Cannabis sativa* plant, constituting up to 40 per cent of its extract. CBD was first identified with other cannabinoids almost 5 decades ago [48]. CBD is a safe compound with a wide range of therapeutic applications, including the treatment of psychiatric disorders [49]. As such, CBD is an attractive candidate for future therapeutic use. There are, however, several caveats to the story. CBD has low and variable oral bioavailability in man [50] and possesses a narrow therapeutic dose range. The development of compounds with a good safety and clinical profile but a wider effective dose range is clearly a priority. In their discussion, Guimarães and co-workers [13] review behaviour studies that clearly indicate that more than one mechanism is involved in CBD action, depending on the effects being measured (anxiolytic, anti-compulsive, antidepressant or antipsychotic-like) and the drug regime (single versus repeated administration). CBD acute anxiolytic-like effects appear to involve facilitation of 5-HT1A-mediated neurotransmission in key brain areas related to defensive responses, including the dorsal periaqueductal grey, bed nucleus of the stria terminalis and medial prefrontal cortex. Anti-compulsive, increased extinction and impaired reconsolidation of aversive memories, facilitation of adult hippocampal neurogenesis and blockade of the anxiogenic consequences of chronic unpredictable stress may depend on potentiation of anandamide-mediated neurotransmission, while activation of TRPV1 channels may be invoked to explain the anti-psychotic effect and the bell-shaped dose-response curves frequently seen with CBD. Additional *in vivo* studies, combined with medicinal chemistry efforts may further elucidate the biochemical pathway(s) underlying CBD behavioural effects while identifying analogues with an improved efficacy profile.

3. THE FUTURE

Despite the public concern related to the abuse of marijuana and its derivatives, research on the ECS has recently aroused enormous interest not only for the physiological functions, but also for the promising therapeutic potentials of drugs modulating the activity of cannabinoid receptors—especially within the nervous system. The brain is considered by many as the last great frontier of science. Indeed, the brain is the 'commander-in-chief' for the entire body, a fascinating and extremely complicated organ in its own right. Disorders of the nervous system as a whole, account for more hospitalizations, more long-term care and more chronic suffering than nearly all other disorders combined. What still remains a fact is that previous years have shown a marked improvement in the treatment of various neurological conditions thanks to the vast advancements in structural and functional neuro-imaging. The answer to all diseases lies at the molecular level and why should the brain be any different? Given the broad involvement of the ECS in nervous system development, homeostasis, dysfunction and energy balance, the more we know of the ECS the better are the prospects for capitalizing on endocannabinoid-based therapies in each disease context.

One needs only look at new therapies which have been or are becoming available which are based on the ECS. Just think of Rimonabant for the treatment of obesity (now discontinued, but research is still ongoing to develop CB1 receptor antagonists with lesser side effects as anti-obesity agents) and Sativex (containing CBD and THC in nearly equal amounts) for the symptomatic relief of neuropathic pain and spasticity in multiple sclerosis. Future studies will no doubt shed light on other applications now developing, including Alzheimer's disease [51], Huntington's disease [52] and as treatment for the emotional processing impairment presented in schizophrenia [53].

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