Review

The biology that underpins the therapeutic potential of cannabis-based medicines for the control of spasticity in multiple sclerosis

David Baker*, Gareth Pryce, Samuel J. Jackson, Chris Bolton, Gavin Giovannoni

Department of Neuroscience and Trauma, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom

Abstract

Cannabis-based medicines have recently been approved for the treatment of pain and spasticity in multiple sclerosis (MS). This supports the original perceptions of people with MS, who were using illegal street cannabis for symptom control and pre-clinical testing in animal models of MS. This activity is supported both by the biology of the disease and the biology of the cannabis plant and the endocannabinoid system. MS results from disease that impairs neurotransmission and this is controlled by cannabinoid receptors and endogenous cannabinoid ligands. This can limit spasticity and may also influence the processes that drive the accumulation of progressive disability.

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1. Introduction

Multiple sclerosis (MS) is an immune-mediated, demyelinating and neurodegenerative disease of the central nervous system (CNS) that affects about 1:500–1:800 people within regions of the United Kingdom (Compston and Coles, 2002, 2008). This chronic disease causes periods of neurological attack and accumulating disability and the development of a number of clinical symptoms such as pain and spasticity (Compston and Coles, 2002; Shakespeare et al., 2003) and this has prompted some people to self-medicate and perceive benefit from taking cannabis (Consroe et al., 1997; Clark et al., 2004). Benefits were reported for some symptoms, notably sleep disturbances, pain, spasms and spasticity (Consroe et al., 1997). At the time of those initial studies, little could people appreciate that an underlying biology and objective experimental evidence that underpins these
perceptions would be subsequently uncovered (Baker et al., 2000; Howlett et al., 2002; Katona and Freund, 2008; Pertwee et al., 2010). This also provided a clear logic for the development of cannabis-based medicines for the treatment of spasticity.

Although the cannabis plant contains many chemicals, Δ⁹-tetrahydrocannabinol (THC) was found to be the main, active ingredient associated with the psychoactive effects of cannabis (Mechoulam and Gaoni, 1967; Howlett et al., 2002; Varvel et al., 2005). Cannabidiol (CBD) is the major non-psychoactive cannabinoid compound of cannabis, which may have medicinal properties and may modify the pharmacobiology of THC to reduce psychoactivity (Russo and Guy, 2006; Pertwee, 2008; Karschner et al., 2011). Although dronabinol®, which is a synthetic THC oral formulation, is licensed as an anti-emetic in cancer chemotherapy, Sativex®, has recently become the first botanical cannabis-based medicine to be licenced, in some European countries, for the treatment of spasticity (Knietowicz, 2010). The therapeutic activity of Sativex occurs because it induces signalling via the endocannabinoid system. The biology of this system is only just being uncovered, but it offers therapeutic potential.

2. The endocannabinoid system

Plant and most synthetic cannabinoids are extremely hydrophobic molecules that rapidly penetrate the CNS and cell membranes (Pertwee, 1999, 2008; Howlett et al., 2002; Pertwee et al., 2010). However, with the advent of synthetic, high affinity cannabinoids it became evident that the cannabinoid molecules were acting via receptor-driven events (Howlett, 1985; Devane et al., 1988). In 1990, the cannabinoid receptor type 1 (CB₁) was cloned (Matsuda et al., 1990) and the cannabinoid system was revealed. The CB₁ receptor is an evolutionarily-conserved, single exon, G/Go G-protein coupled receptor, which is the most abundant G-protein coupled receptor in the CNS (Howlett et al., 2002). This receptor is coupled to adenylate cyclase and a number of calcium channels and inward rectifying potassium channels (Howlett et al., 2002; Guo and Ikeda 2004). The CB₁ receptor is expressed by all nerve subtypes, but is abundant in the basal ganglia, cerebellum and areas associated with balance, but is weakly expressed in the brainstem, which contains areas controlling vital functions (Howlett et al., 2002). The CB₂ receptor is also expressed in the hippocampus, which is associated with short-term memory formation (Herkenham et al., 1990; Howlett et al., 2002). The range of CB₁ receptor expression therefore began to explain cannabis intoxication, where disturbances in balance and short-term memory processing were evident and the fact that cannabis and THC overdose, in contrast to other recreational drugs, do not have a significant risk for mortality (Howlett et al., 2002). Although the CB₁ receptor is abundantly expressed in CNS tissue, it is found also in a number of non-CNS tissues including the dorsal root ganglion cells (DRG) and the neuromuscular junction (Fig. 1, Howlett et al., 2002), such that it is strategically located in sensory and motor pathways of neurotransmission that are affected during MS (Compston and Coles, 2002, 2008). Therefore cannabinoids can influence symptoms at both peripheral and central sites within the nervous system.

CB₁ receptors are also expressed by immune cells, such as macrophages, polymorphonuclear neutrophils and lymphocytes (Howlett et al., 2002). However, cells of the immune system notably express the CB₂ receptor (Howlett et al., 2002). This is only about 50% homologous to the CB₁ receptor and also signals via adenylate cyclase but lacks the ionotropic signalling of the CB₁ receptors (Howlett et al., 2002). This probably serves to regulate the degree of immune activation, but the function of this receptor remains poorly understood.

The discovery of the cannabinoid receptors has facilitated the production of many cannabinoid receptor binding compounds of varied structure, termed cannabinoids, including the discovery of fatty acid, endogenous ligands, termed endocannabinoids (Pertwee, 1999; Howlett et al., 2002; Devane et al., 1992; Mechoulam et al., 1995). The first ligand discovered was arachidonoyl ethanolamide

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**Fig. 1.** Routes for cannabinoid control of symptoms of multiple sclerosis and spinal injury. Following injury to the brain and spinal cord, problems of neurotransmission may occur that lead to the development of pain, spasticity and bladder problems. Although these events are triggered by CNS disease, sensory nerves transmit signals concerning sensation and positional cues from tissues to the brain and motor responses are transmitted from the brain to the tissues. Although aberrant signalling can be controlled at the level of motor and sensory pathways within the brain and spinal cord, in injury, spinal reflex arcs may form to compensate for nerve transactions within the CNS. Cannabinoid receptors are strategically located throughout the CNS and also in afferent and efferent nerve pathways in the peripheral nervous system and at the neuromuscular junction, which may be targeted to induce therapy, whilst avoiding cannabinoid receptor-mediated impairment of cognitive function in the brain.
(AEA), also called anandamide (Devane et al., 1992) and this was followed by the discovery of 2-arachidonoyl glycerol (2-AG (Mechoulam et al., 1995)). Multiple pathways are involved in the generation of anandamide and 2-AG which are produced on-demand in synaptic membranes (Basavarajappa, 2007; Di Marzo, 2008). Anandamide can be generated from its membrane phospholipid precursor N-arachidonoyl phosphatidylethanolamine (NAPE) through hydrolysis by a phospholipase D (NAPE-PLD) (Simon and Cravatt, 2006, 2008; Basavarajappa, 2007; Di Marzo, 2008; Liu et al., 2008). However NAPE-PLD deficient mice did not have altered anandamide levels suggesting other pathways can also generate anandamide synthesis (Leung et al., 2005; Tsuboi et al., 2011). One pathway involves sequential deacylation of NAPE by alpha,beta-hydrolase 4 (ABH4) and the subsequent cleavage of glycerophosphate to yield anandamide (Di Marzo, 2008; Simon and Cravatt, 2006). Another pathway proceeds through phospholipase C-mediated hydrolysis of NAPE to yield phosphoanandamide, which is then dephosphorylated by a number of phosphatases (Leung et al., 2006; Basavarajappa, 2007; Di Marzo, 2008). Similarly, 2-AG may be formed by multiple pathways and requires consecutive activation of two distinct enzymes (Basavarajappa, 2007; Di Marzo, 2008; Jung et al., 2007; Ueda et al., 2011). A phospholipase A2 (PLA2) hydrolyses phosphoinositols precursors to produce a lyso 2-arachidonoyl phosphoinositol (LAPL) and hydrolysis of this via lysophospholipase C (LPLC) can also produce 2-AG (Basavarajappa, 2007; Di Marzo, 2008; Jung et al., 2007). However it is clear that a phospholipase C (PLC) catalyzes formation of the 2-AG precursor, 1,2-diacylglycerol (DAG) from membrane phosphoinositides and subsequently diacylglycerol lipase (DAGL) alpha catalyzes hydrolysis of 1,2-diacylglycerol to generate 2-AG (Tanimura et al., 2010; Gao et al., 2010). The consequences of specific upregulation of anandamide and 2-AG synthesis in control of spasticity have yet to be assessed in MS. However, the complex nature of endocannabinoid production may limit the druggability of these targets.

In contrast, many drugs have been developed and used experimentally that block endocannabinoid degradation. This was believed to involve a two stage process. Anandamide and 2-AG enter the cell via a putative, diffusion-facilitated transporter(s) within the cell membrane, (Beltramo et al., 1997; Pertwee, 1999; Hermann et al., 2006; Di Marzo, 2008; Fu et al., 2011). The endocannabinoids are then hydrolysed to arachidonic acid and water via the actions of specific enzymes. Fatty acid amide hydrolase (FAAH) is an enzyme that recognizes and inactivates anandamide and 2-AG (Di Marzo, 2008). However, in vivo, it appears to function as the degrading enzyme of anandamide only, because the pharmacological and genetic inactivation of fatty acid amide hydrolase is usually accompanied by elevation of anandamide, but not 2-AG, levels (Di Marzo, 2008; Deutsch et al., 2002; Cravatt and Lichtman, 2002; Lichtman et al., 2002). The major enzyme responsible for 2-AG hydrolysis is monoacylglycerol lipase ( MAGL) (Dinh et al., 2002; Blankman et al., 2007). This accounts for about 85% of 2-AG degradation, whereas fatty acid amide hydrolase only accounts for just 1% of 2-AG degradation (Blankman et al., 2007). It has been reported that alpha beta hydroxylase 12 accounts for 9% of 2-AG degradation and alpha beta hydroxylase 6 accounts for 3% of 2-AG degradation and are important mediators of 2-AG degradation at luminal and cytosolic sites in the membrane, respectively (Blankman et al., 2007; Marrs et al., 2010). Their biological significance will become better uncovered with the development and use of specific inhibitory drugs and the genetic inactivation of the pertinent molecules.

The functions of the endocannabinoid system are slowly being uncovered, but the finding that the endocannabinoids/CB1 receptor system regulates synaptic neurotransmission (Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001; Howlett et al., 2002; Katona and Freund, 2008; Tanimura et al., 2010) provides a very strong rationale for cannabinoid control of pain, spasticity and all other neurological symptoms (Fig. 2). These symptoms result from loss of homeostatic control of neurotransmission, due to disease-related damage to the neural circuitry (Figs. 2 and 3).

3. The neural function of the endocannabinoid system

Neurotransmitter release across synapses stimulates postsynaptic, ionic fluxes that generate action potentials to propagate neural signalling. This can lead to endocannabinoid release from...
in and around the synaptic membrane (Nyilas et al., 2008). These act as retrograde synaptic messengers to stimulate pre-synaptic CB$_1$ receptors that quell further pre-synaptic activity (Fig. 2, Howlett et al., 2002; Katona and Freund, 2008; Lúdányi et al., 2011). Depolarization induced suppression of inhibition (DSI) in inhibitory, gamma aminobutyric acid (GABA) neurons and depolarization induced suppression of excitation (DSE) in excitatory, glutamatergic neurons transiently, within 1–10 s, curtails further pre-synaptic neurotransmitter release following post-synaptic depolarization and is mediated by the action of endocannabinoids (Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001; Howlett et al., 2002; Diana and Marty, 2004; Katona and Freund, 2008). Endocannabinoids can also trigger longer-term synaptic plasticity and facilitate Long-term depression (LTD), which is a lasting decrease in synaptic effectiveness that follows some types of electrical stimulation (Howlett et al., 2002; Katona and Freund, 2008). This could be usefully stimulated following the development of chronic aberrant neurotransmission during disease. Excessive glutamate can lead to stimulation of metabotropic glutamate receptors outside the synaptic cleft or ionotropic...
glutamate receptors within the synapse leading to excessive intracellular Ca$^{2+}$, which leads to endocannabinoid release from the membrane (Maejima et al., 2001; Drew et al., 2008; Katona and Freund, 2008) (Fig. 2).

The current pharmacology suggests that 2-AG is the important retrograde inhibitor of synaptic neurotransmission relating to DSI and DSE (Szabo et al., 2006; Hashimoto-dani et al., 2008; Jung et al., 2007; Hashimoto-dani et al., 2008; Pan et al., 2009; Tanimura et al., 2010). As inhibitors of anandamide degradation also affect cannabinoid-mediated control of neural signalling, this suggests that anandamide may likewise control neurotransmission (Wilson and Nicoll, 2002; Howlett et al., 2002; Nyilas et al., 2008). The CB1 receptor normally exists in a partially pre-coupled state that can allow for rapid upregulation or downregulation of signalling (Pertwee, 1999; Howlett et al., 2002). As 2-AG has a lower affinity for the CB1 receptor and is significantly more (about 100–1000 fold) abundant than anandamide (Pertwee, 1999; Baker et al., 2001; Howlett et al., 2002), it is possible that 2-AG provides this low level stimulation of the CB1 receptor (Howlett et al., 2011) in order to maintain homeostatic CB1 receptor activity and DSI/DSE. Anandamide, which has a higher affinity for CB1 receptor than 2-AG (Pertwee, 1999) and is rapidly produced following injurious stimuli (Schabitz et al., 2002; Patel et al., 2005; Centonze et al., 2007) may be more important in regulating nerve function in pathological conditions. This would suggest that the anandamide pathway may be a good target in inhibiting disease. This is further supported by the recent observation that inhibition of MAG lipase, which enhances 2-AG levels, induces marked cannabimimetic effects akin and cannabinoid receptor tolerance to CB1 receptor agonism (Long et al., 2009; Scholzburg et al., 2010), in contrast to the low cannabimimetic effect-inducing potential of anandamide degradation inhibitors. This indicates that up-regulation of the 2-AG pathway will be of limited use as a therapeutic target. However, it has been suggested that in some situations FAAH inactivation in specific brain areas could increase 2-AG concentrations, whereas in others the FAAH-induced elevation of anandamide levels will cause a reduction of 2-AG biosynthesis via transient resting potential vanilloid one (TRPV1) receptor stimulation (Maccarrone et al., 2008; Di Marzo and Maccarrone, 2008).

The finding that endocannabinoids can regulate synaptic neurotransmission (Fig. 2) provides a clear rational why stimulation of CB1 receptors by cannabis-based drugs will have the potential to regulate the aberrant level of glutamatergic excitability during spasticity (Brown, 1994, Fig. 3). However, the biology of disease is complicated and the outcome of cannabinoid receptor stimulation or inhibition of endocannabinoid degradation may either be: excitatory, inhibitory or disinhibitory. This will depend on the location of the cannabinoid receptor, the endocannabinoid precursors and endocannabinoid degradation machinery within the affected neural circuitry. Therefore, treatments aimed at the cannabinoid system are likely to be able to induce both positive and negative effects as is evident following administration of marijuana (Howlett et al., 2002). However, during multiple sclerosis and neuroinflammation, GABAergic inhibitory neurons may be lost and glutameric hyperexcitability appears to predominate and may contribute to the development of signs of disease and neurodegeneration (Smith et al., 2000; Dutta et al., 2006; Centonze et al., 2007; Rossi et al., 2009). Therefore limitation of excessive glutamatergic signalling using agents by cannabinoids may limit symptoms of MS and the development of progressive disease (Fig. 3).

4. Pathophysiology of spasticity

Spasticity is the uncontrolled limb function that results from damage to the nervous system, notably the descending motor pathway of the corticospinal tract, which controls voluntary movement (Brown, 1994; Nielsen et al., 2007). This damage results in muscle spasms, clonus (a series of rapid muscle contractions) and hypertonia (increased muscle tone), which interfere with movement and range from mild stiffness to painful, uncontrollable spasms. This condition is often associated with diseases including: multiple sclerosis, spinal cord injury, amyotrophic lateral sclerosis, cerebral palsy and brain damage (Brown, 1994; Adams and Hicks, 2005). Spasticity develops over several months following the primary lesion and involves adaptation in the spinal neuronal circuitries caudal to the lesion and may be due to a reduction of spinal inhibitory mechanisms. Under normal circumstances, inhibitory signals are sent via the corticospinal tract to the spinal cord, but following injury, damage to the corticospinal tract causes disinhibition of the stretch reflex leading to a reduction in the triggering threshold. This results in excessive contraction of the muscles, sometimes even at rest (Brown, 1994; Nielsen et al., 2007). Therefore treatment of spasticity is targeted with drugs aimed at influencing neurotransmitter actions within the corticospinal tract. Baclofen is a GABA$_B$ receptor agonist that is one of the first-line agents used in the treatment of spasticity (Shakespeare et al., 2003). Stimulation of GABA receptors increases transmembrane potassium conductance through specific ion channels and this has an inhibitory, hyperpolarizing effect on the resting electrochemical potential of the nerve. This typically decreases the rate of neuronal action potentials thus limiting hyperexcitation of the muscles (Brown, 1994; Adams and Hicks, 2005; Nielsen et al., 2007). Other agents such as dantrolene act by abolishing excitation-contraction coupling in muscle cells (Shakespeare et al., 2003), suggesting that some elements of spasticity may be controlled in the periphery (Fig. 1) as has been found in some pain paradigms (Agarwal et al., 2007; Dzidzulewicz et al., 2007). The perception that cannabis can help alleviate spasticity and spasms (Consroe et al., 1997), has become underpinned by increasing understanding of the biology of the cannabinoid system and by experimental evidence in models of spasticity.

5. Animal models of multiple sclerosis

Whilst multiple sclerosis is clearly a uniquely human disease, the chief pathological hallmarks of multiple sclerosis are: mononuclear infiltration of the CNS, demyelination and nerve damage that results in altered and aberrant neurotransmission and the development of neurological signs (Compston and Coles, 2002, 2008). Elements of these features can all be modelled in animals (Baker et al., 1990, 2000; Pryce et al., 2005; Baker and Jackson, 2007; Maresz et al., 2007; Croxford et al., 2008; Al-Izki et al., 2009). Experimental autoimmune encephalomyelitis (EAE) is a model of MS, which can be induced in many mammalian species such as rodents and non-human primates following immune sensitization to CNS, typically myelin, antigens (Baker et al., 1990; Baker and Jackson, 2007). Although EAE is largely used to study immune function, other features of MS can be replicated in animals, if disease is studied for sufficient time (Baker et al., 2000; Pryce et al., 2005; Al-Izki et al., 2009).

Whilst, it has been argued by some that animal (EAE) studies may not be useful for identifying drugs for the treatment of MS (Sirram and Steiner, 2005; Ransohoff, 2006), it must be accepted that without the knowledge generated from animal and other non-clinical studies, no useful MS therapies would be available (Baker et al., 2011) Although differences in biology between human and rodents exist and can account for some failure to translate EAE studies into the treatment of MS, this is particularly facilitated by belief in the myth that all the problems of MS are
solely the result of autoimmunity, which can be treated with non-immunological therapeutics (Baker and Jackson, 2007; Baker et al., 2011). However, it is becoming increasingly clear that whilst anti-immunological agents make a dramatic impact (> 60–80% inhibition) on relapses if treatment is initiated early in disease course (Polman et al., 2006; CAMMS223 Trial Investigators et al., 2008; Giovannoni et al., 2010), such immunosuppressive agents do not inhibit the accumulation of progressive disability once autoimmunity has generated a neural environment that is conducive to the development of neurodegeneration (Coles et al., 1999; Rice et al., 2000; Compton and Coles, 2002; Dutta and Trapp, 2007). This is also the case in animals as well as humans (Pryce et al., 2005; Hampton et al., 2008; Al-Izki et al., 2011), but it had not been appreciated by many as most animal experiments in EAE are concluded within a few weeks, yet progressive EAE can take months to develop (Pryce et al., 2005; Al-Izki et al., 2011). However once progressive nerve loss has been accumulated the underlying problems of aberrant neurotransmission and loss of neural circuitry are common features to both animals with EAE and humans with MS. Therefore, it is likely that the pathophysiology of spasticity is similar in animals and humans. Therefore animals can be used to assess anti-spastic activity of drugs, such as cannabinoids.

6. Direct and indirect CB1 receptor agonism inhibits experimental spasticity

Although spasticity is a common problem experienced in multiple sclerosis and spinal cord damage, there are few experimental models of spasticity in which experimental therapies can be investigated (Baker et al., 2000; Oshiro et al., 2010). Spasticity has been reported to develop in animals after repeated paralytic attacks and the accumulation of nerve damage, months after the induction of EAE (Baker et al., 2000, 2001; Jackson et al., 2005). Based on the concept that symptoms of multiple sclerosis are caused by aberrant neurotransmission, there is a clear mechanistic rationale for cannabinoids to control signs of disease (Figs. 2 and 3). Cannabinoid (CB1) receptor agonism using cannabis and synthetic cannabinoids ameliorates limb and tail spasticity in the presence and importantly in the absence of obvious cannabimimetic effects that are associated with high dose cannabinoids (Baker et al., 2000, 2001, 2004; Wilkinson et al., 2003; Pryce and Baker, 2007, Unpublished observation). Despite some promise of activity of CB2 receptor agonists as anti-spastic agents (Baker et al., 2000), it was found that their inherent cross-reactivity with CB1 receptors (Pertwee, 1999) appeared to be the mode of therapeutic activity (Pryce and Baker, 2007). Furthermore the anti-spasticity activity of potent CB1/CB2 receptor agonists was lost in CB1 deficient mice, it clearly demonstrates that the CB1 receptor is central to the therapeutic activity of cannabinoids (Pryce and Baker, 2007). This provides objective evidence for the potential of cannabinoids for the control of spasticity in multiple sclerosis.

Whilst cannabinoid receptor agonism alleviated limb and tail spasticity in rodent models, most importantly, cannabinoid receptor antagonism transiently worsened the frequency/incidence of spasticity and tremor (Baker et al., 2001; Brooks et al., 2002). This suggested tonic control of spasticity by the endocannabinoid system and further supports the value of cannabinoids for symptom control in MS. The endocannabinoid levels are dysregulated in areas exhibiting pathology in EAE and MS tissues (Baker et al., 2001; Eljaschewitsch et al., 2006; Centonze et al., 2007). Analysis of endocannabinoids levels indicated a significant increase of anandamide and 2-AG in areas of nerve damage and EAE-spinal cord lesions in spastic animals compared to non-spastic, diseased animals (Baker et al., 2001). This suggests that the endocannabinoid system is upregulated in lesional areas to provide further control of aberrant neurotransmission and suggested that further enhancement of endocannabinoid tone by stimulating endocannabinoid synthesis or blockade of endocannabinoid degradation may exhibit anti-spastic activity. Indeed, inhibitors of the putative anandamide re-uptake mechanism have ameliorated spasticity (Baker et al., 2001; de Lago et al., 2004, 2006; Ligresti et al., 2006). However, the re-uptake transporter has yet to be cloned and there has been recent evidence questioning the existence of a specific re-uptake transporter (Glaser et al., 2003; Di Pasquale et al., 2009). In particular, the prototypic transport inhibitor, AM404, has been reported by some to have CB1 receptor binding affinity, transient receptor potential vanilloid receptor (TRPV1) agonist activity and in particular be a fatty acidamide hydrolase inhibitor (Beltramo et al., 1997; Jarrahian et al., 2000; Ralevic et al., 2001). Each of these could contribute to therapeutic activities of putative transport inhibitors in spasticity (Baker et al., 2001; Brooks et al., 2002) and pain (Jayamanne et al., 2006; Wang, 2008). However, AM404 increases anandamide levels in FAAH-deficient mice (unpublished observations) suggesting an activity independent of FAAH inhibition. Therefore, if a specific transport molecule does not exist as is becoming increasingly likely, these agents probably act competitively to allosterically inhibit biochemically-compatible sites of anandamide diffusion within the plasma membrane, as has been reported for interference in some receptor systems (Barann et al., 2002). However, as the full extent of the endocannabinoid system is evolving (Pertwee et al., 2010) and the identity of additional endocannabinoid receptors may be currently unknown, the tools (specific antagonists, and gene knockout mice) have yet to be generated to prove the involvement of most cannabinoid molecules in the disease process. Reagents to probe the 2-AG pathway have been generated and monoacylglycerol lipase inhibitors can inhibit spasticity (Comelli et al., 2007, Unpublished observations). Perhaps as the level of anandamide is a thousand fold less than the occurrence of 2-AG, degradation inhibitors of anandamide are not associated with the induction of cannabimimetic effects that can occur following inhibition of 2-AG degradation by inhibition of MAG lipase (Pertwee, 1999; Long et al., 2009). This suggests that the pharmacological blockade of FAAH to promote anandamide levels may have therapeutic potential. Although FAAH-selective drugs can have off-target effects, the use of fatty acidamide hydrolase knockout mice provides conclusive evidence that fatty acidamide hydrolase is a potential target for analgesia (Lichtman et al., 2002; Cravatt et al., 2002; Ahn et al., 2011) and spasticity (Baker et al., 2001, Unpublished observations). Whilst it remains to be established whether FAAH inhibitors will have clinical utility in the control of spasticity in MS, importantly it further indicates that the cannabinoid system controls spasticity and that exogenous CB1 receptor agonism should inhibit spasticity.

7. Tetrahydrocannabinol is the major therapeutic cannabinoid for spasticity

The cannabis plant contains a variety of chemical substances, whose biological activities have yet to be investigated fully. As a cannabis extract (extracted via alcohol) appeared to perform better than pure THC in the treatment of spasticity in EAE (Wilkinson et al., 2003), this could suggest that there could be some advantage of exploiting the botanical compared to chemical synthesis route for the production of medicinal cannabinoids. However, synthetic CB1 receptor agonists and endocannabinoid degradation inhibitors can perform as least as well as THC and cannabis in experimental spasticity (Baker et al., 2000; Wilkinson et al., 2000).
et al., 2003; Pryce and Baker, 2007). However, the presence of THC within the cannabis extract was central to the therapeutic effect, as removal of THC from a Mexican cannabis extract (lacking CBD), rendered it devoid of therapeutic activity (Wilkinson et al., 2003). There is currently no plausible evidence to suggest that CBD can interfere with the mechanisms involved in the pathophysiologyst of spasticity and this is consistent with the lack of activity against experimental spasticity (Baker et al., 2000). The presence of cannabidiol within the cannabis extract, creates a commercial and intellectual property difference between Sativex and synthetic THC (Marinol/dronabinol), but as it is not inert (Izzo et al., 2009), the cannabidiol may have therapeutic potential in other aspects of the disease. However, it has been reported that CBD can influence the pharmacokinetic profile and biological activities of THC (Hollister and Gillespie, 1975; Russo and Guy, 2006; Varvel et al., 2006; Hayakawa et al., 2008; Karschner et al., 2011). Indeed it has been reported that CBD can antagonise some of the undesirable effects of THC including intoxication and sedation while contributing therapeutic properties in its own right (Russo and Guy, 2006). As such it has been argued that CBD may have CB1 and CB2 receptor antagonistic properties (Thomas et al., 2007; Pertwee, 2008), which would be expected to limit adverse cannabimimetic effects. This could also limit the therapeutic effects of THC in experimental spasticity. However, such antagonism was not evident in mice with spasticity, as CBD failed to augment spasticity in contrast to that seen with Rimonabant/SR141716A (Baker et al., 2000). Furthermore at 1:1 ratios, CBD did not inhibit the cannabimimetic effects of THC in mice (Varvel et al., 2006). This indicates in vivo that CBD is not exhibiting overt CB1 receptor antagonism, therefore it is inconceivable that doses of Sativex botanical drug substances would not be found that inhibit spasticity in experimental EAE, which is indeed the case (Patel et al., 2010) (Fig. 4).

Although THC and CB1 receptors clearly mediate the therapeutic effects (Pryce and Baker, 2007), it is also event that THC and CB2 receptors are responsible for many of the adverse effects of cannabis in both humans and rodents (Huestis et al., 2001, 2007; Howlett et al., 2002; Varvel et al., 2005). Therefore, the clinical utility of medical cannabis extracts is going to be a balance between positive and adverse effects, as is the case with current anti-spasticity agents used for multiple sclerosis (Shakespear et al., 2003). It also indicates that optimal doses of CB1 receptor agonism in motor control centres would invariably be associated with stimulation of CB1 receptors in cognitive centres, which could be associated with some unwanted side effects. Therefore the therapeutic window of effects verses side-effects of CNS-penetrant CB1 receptor agonists, including THC is therefore going to be narrow, in contrast to that seen with experimental CNS-excluded CB1 receptor agonists (Baker et al., 2004; Dyson et al., 2005; Dziadlewicz et al., 2007) and anandamidine degradation inhibitors (Baker et al., 2001; de Lago et al., 2004, 2006; Ligresti et al., 2006; Boiger et al., 2000) (Unpublished observations) that have a lower propensity to induce cannabimimetic effects. Animals lack the mental and vocal capabilities of humans and therefore it is not possible to truly assess the adverse/psychoactive actions of cannabinoids in rodents. The cannabimimetic effects of chemicals in rodents are detected by behavioural and often motor outcomes, that probably lack the subtlety of effects that a human may perceive (Howlett et al., 2002; Varvel et al., 2006). Whether a drug works in humans will depend upon tolerability of the drug and whether there is sufficient neural circuitry for the drug to act upon at the time of treatment. However, therapy with THC and cannabis, without obvious cannabimimetic effects is feasible in animals (Baker et al., 2000; Howlett et al., 2002). This appears to be the case in humans also (Iskedjian et al., 2007; Collin et al., 2007; Rog et al., 2007).

8. Targeting of cannabinoid compounds to lesions

Although THC clearly penetrates into the CNS (Varvel et al., 2005) the passage of both THC and CBD into the CNS is influenced by drug-exclusion drugs. Drugs entering the CNS will typically diffuse or be transported across the CNS endothelial barriers, but most are excluded by the physiochemical properties of the blood:brain barrier. Polar compounds may not penetrate the lipophilic cell membranes of the endothelia, whereas others are actively excluded from entering the CNS by the action of adenosine triphosphatase (ATP) binding cassette (ABC) transporters such as ABCB1 (multidrug resistance (MDR) p-glycoprotein), ABCG1 (BCRP1-Breast cancer resistance protein one) and ABCG1 (MRP1-multidrug resistance protein one) that notably exclude hydrophobic compounds and includes cannabinoids (Zhu et al., 2006; Holland et al., 2007, 2008; Bonhomme-Faivre et al., 2008). CBD treatment can influence both ABCG2 function (Holland et al., 2007) and penetration of THC into the brain (Bornheim et al., 1995). Importantly however is the observation that THC behaves as a substrate for p-glycoprotein/ABCB1 in vivo and would limit entry of THC into the CNS (Bonhomme-Faivre et al., 2008). During both EAE and MS the activity of some of these pumps, including ABCB1 is lost from lesions (Kooij et al., 2010). The loss of p-glycoprotein could lead to at least a two fold increase of THC entry into the CNS. Therefore THC will be selectively delivered to lesions, which are concentrated in the motor centres in the brain and spinal cord. Therefore, more CB1 receptor agonism can be delivered to facilitate symptom control and importantly deliver neuroprotection to the sites of damage.

9. Clinical experience of cannabis-based medicines

Although studies are not universally positive (Centonze et al., 2009), larger placebo controlled trials in multiple sclerosis have
shown a small but positive subjective improvement in MS (Zajicek et al., 2003; Wade et al., 2006; Collin et al., 2010; Novotna et al., 2011) and cannabis-based medicines have been approved for treatment of symptoms of MS in some countries. The first large scale trials of botanical cannabis based medicines demonstrated that whilst cannador an orally-delivered mixture of CBD and THC, did not influence the primary outcome measure which was the objective, but insensitive Ashworth scale of spasticity (Zajicek et al., 2003). Subjective patient-orientated outcomes of spasticity were consistently shown to respond to THC and cannabis-based medicines (Zajicek et al., 2003, 2005; Zajicek and Apostu, 2011). Likewise subjective outcomes of spasticity were found to respond to Sativex (Wade et al., 2006, 2010; Collin et al., 2010; Novotna et al., 2011; Notcutt et al., 2011). However, it was evident that a significant proportion of people with MS did not respond to treatment (Novotna et al., 2011). This could relate to the differences of individuals to respond to treatment but it is possible that the disease had advanced such that the neural circuitry capable of responding to treatment, had been lost due to disease progression. These studies indicate that there is a narrow therapeutic window between efficacy and the induction of mild cannabimimetic effects. In addition to control of spasticity cannabinoids have influenced other symptoms of MS that include chronic pain (Rog et al., 2005, 2007; Iskedjian et al., 2007; Conte et al., 2009) and a small influence on bladder over-activity in some instances (Kavia et al., 2010). Likewise although Cannador failed to show an influence on bladder function is some studies (Zajicek et al., 2003), they exhibited positive in another study (Freeman et al., 2006). Therapy with Sativex was not associated with cannabinoid receptor tolerance to stimulation (Robson, 2011). Furthermore at therapeutic doses Sativex did not induce overt psychopathology, although street cannabis has been associated with reduced cognitive performance (Aragona et al., 2009; Robson, 2011; Honarmand et al., 2011). Therefore cannabis-based medicines offer potential to control spasticity in MS.

10. Control of progressive neurological disability in addition to symptom control

Whilst cannabinoids are being considered for symptomatic control of MS, experimental studies suggest that cannabinoids could exert positive influence on other aspects of the disease process. Whilst it has been argued that cannabidiol may have immune-modulating effects (Izzo et al., 2009; Kozela et al., 2011), this was not demonstrated in early stage EAE (Maresz et al., 2007). Likewise, although repeated bolus of endocannabinoids, which are presumably degraded rapidly by hydrolytic enzymes in organs such as the liver, have been reported to inhibit the development of autoimmunity (Bittner et al., 2009; Lourbopoulos et al., 2011), this seldom occurs with cannabinoide receptor agonists at doses that are not associated with significant cannabimimetic effects (Maresz et al., 2007; Croxford et al., 2008; Hasseldam and Johansen, 2010). Tetrahydrocannabinol can, with proviso, inhibit the development of immune processes that drive paralytic attacks in animal models of neuroimmunological damage (Lyman et al., 1989; Maresz et al., 2007; Croxford et al., 2008). Suppression of autoimmunity by THC is probably an indirect effect of stimulation of release of immunosuppressive chemicals following CB1 receptor mediated signalling from brain centres (Maresz et al., 2007; Croxford et al., 2008). Furthermore CB2 receptor stimulation can inhibit immune function and the generation of EAE (Maresz et al., 2007; Palazuelos et al., 2008; Zhang et al., 2009; Mestre et al., 2009). However the immunosuppressive effect of THC occurs at a high dosage via a CB2 dependent mechanism (Maresz et al., 2007), which is probably not achievable clinically. This would be consistent with the use of Marinol® for the treatment of acquired immunodeficiency syndrome, where immunosuppression would be contra indicated, and the relative lack of changes in immune phenotype have been detected in studies using cannabis in humans (Rachelefsky et al., 1976; Killestein et al., 2003; Katona et al., 2005).

However, more important was the observation that the objective signs of spasticity were improved following long-term treatment with oral THC (Zajicek et al., 2005). This suggested that cannabinoids may slow the disease progression or promote repair and compensation for neurological deficits. Indeed, these aspects are all supported in experimental models. Demyelination and low grade microglial inflammation leaves nerves vulnerable to damage and death via metabolic failure, loss of trophic support and excitotoxicity (Smith et al., 2000, 2001; Dutta et al., 2006; Dutta and Trapp, 2007). These processes accumulate in toxic levels of intracellular Ca2+, which can be limited by the process that quell symptoms (Fig. 3) are typical of many neurodegenerative conditions such as: stroke, trauma, and motor neuron disease where cannabinoids could have neuroprotective effects (Pryce et al., 2003; Nagayama et al., 1999; Panikashvili et al., 2001; Parmentier-Batteur et al., 2002; Bilsland et al., 2006; Kim et al., 2006; Docagne et al., 2007; Croxford et al., 2008; Hasseldam and Johansen, 2010, 2011). Enhanced levels of endocannabinoids in quiescent, chronic, spastic disease (Baker et al., 2001) not only provides a mechanism for control of excessive neurotransmission, but may also provide neuroprotection from excessive excitotoxicity as has been suggested to occur in MS tissue (Eilaschewitsch et al., 2006; Centonze et al., 2007; Loría et al., 2010; Rossi et al., 2011). Likewise the reduced endocannabinoid levels during active paralytic disease has been attributed to the development of neurodegeneration that occurs as a consequence of the attack (Cabranes et al., 2005; Witting et al., 2006). This would be consistent with the observations that CB1 deficient mice poorly tolerate excitotoxic and immune attacks to the CNS and rapidly develop significant nerve loss (Pryce et al., 2003; Jackson et al., 2005). In contrast FAAH-deficient mice and CB1/CB2 receptor agonist treated animals exhibit improved recovery from immune attack and less nerve loss than wildtype and placebo treated mice, respectively (Croxford et al., 2008; Webb et al., 2008; Rossi et al., 2011). Furthermore, in addition to limiting autoimmune dependent neurodegeneration (Pryce et al., 2003; Croxford et al., 2008) cannabinoid receptor agonism can protect against neurodegeneration that is not dependent on autoimmunity. (Nagayama et al., 1999; Pryce et al., 2003; Bilsland et al., 2006; Kim et al., 2006). Although CB1 receptors can probably control the elements of neurodegenerative stimuli involving neural processes, CB2 receptors particularly can influence microglial migration and activation (Arévolo-Martín et al., 2003; Klegers et al., 2003; Franklin and Stella, 2003; Mestre et al., 2009), which are central components of the inflammatory process that support nerve loss in a variety of neurodegenerative conditions. As such CB2 receptor agonist treatment has the potential to slow neurodegenerative disease as found in a model of motor neuron disease (Kim et al., 2006). Tetrahydrocannabinol has been shown to induce neuroprotective effects in animal models of MS via cannabinoid receptors (Pryce et al., 2003). However here additional benefit may be provided by cannabidiol, which has mediates neuromodulation by non-cannabinoid receptor mechanisms (Hampson et al., 1998; El-Remessy et al., 2003; Hayakawa et al., 2007; Izzo et al., 2009) and thus the mixture of cannabinoids within Sativex may promote slowing of progression in MS.

Alternatively to slowing progression of disease, cannabinoids may promote recovery through stimulation of repair processes of endogenous glial cells or by generating neural progenitor cells. This is because both the endogenous cannabinoid system and the...
exogenous stimulation of cannabinoid receptors can promote neural cell development (Palazuelos et al., 2008; Rubio-Araiz et al., 2008; Oudin et al., 2011) and synaptogenesis, which may allow compensatory neural circuits to be developed (Tagliaferro et al., 2006; Chevaleyre et al., 2006; Berghuis et al., 2007; Rossi et al., 2009). Therefore cannabis-based medicines have the capacity to mediate benefit in addition to symptom control. Clinical trials investigating the influence of oral THC on progressive MS are ongoing.

Conflict of interest

Studies involving Sativex botanical substances were supported by GW Pharma Ltd. Patents concerning cannabinoid treatment of spasticity have been filed by DB, GP and GG.

References


