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Mechanisms and Pharmacology of Neuropathic Pain in Multiple Sclerosis

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Abstract

The neuropathic pain of multiple sclerosis is quite prevalent and severely impacts quality of life. A few randomized, placebo-controlled, blinded clinical trials suggest that cannabis- and anticonvulsant-based treatments provide partial pain relief, but at the expense of adverse events. An even smaller, but emerging, number of translational studies are using rodent models of experimental autoimmune encephalomyelitis (EAE), which exhibit pain-like behaviors resembling those of MS patients. These studies not only support the possible effectiveness of anticonvulsants, but also compel further clinical trials with serotonin-norepinephrine reuptake inhibitors, the immunosuppressant drug rapamycin, or drugs which interfere with glutamatergic neurotransmission. Future behavioral studies in EAE models are essential towards a new pharmacotherapy of multiple sclerosis pain.

Keywords

multiple sclerosis; pain; experimental autoimmune encephalomyelitis

1. Introduction

Multiple sclerosis (MS) is an autoimmune-inflammatory neurodegenerative disease of the central nervous system (CNS) that disrupts the myelin sheath, leading to dysfunction of the brain, spinal cord and optic nerves. Worldwide, over 2 million people are affected by MS. It is the most common cause of acquired disability in young adults, usually presenting itself at 20–45 years of age (Ragonese et al., 2008). It is significantly more common in women, with an overall female:male prevalence ratio ranging from 2.6:1 in the United States (Schwendimann and Alekseeva, 2007) to 4:1 in Canada.

The four accepted subtypes of MS are: relapsing and remitting, secondary progressive, progressive relapsing, and primary progressive, and we provide here definitions from Lublin and Reingold (Lublin and Reingold, 1996). The **relapsing-remitting** subtype is present in 85% of patients (McDonald et al., 2001) and is defined as “clearly defined relapses with full recovery or with sequelae and residual deficit upon recovery; periods between relapses characterized by a lack of disease progression”. **Secondary progressive** MS develops in 65–80% of patients previously affected by the relapsing-remitting form, and is defined as

“initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus”. (Lublin and Reingold, 1996) **Progressive relapsing** MS is present in 5–25% of MS patients (Lublin and Reingold, 1996) and is defined as “progressive disease from onset, with clear acute relapses, with or without full recovery; periods between relapses characterized by continuing progression”. **Primary progressive** MS is present in 15% of patients (Cottrell et al., 1999) and is defined as “disease progression from onset with occasional plateaus and temporary minor improvements allowed”.

Pain is a frequent and debilitating feature of MS (Beard et al., 2003, Svendsen et al., 2005, Kumpfel et al., 2007). Two recent systematic reviews indicated that pain is estimated to be present in nearly 50% (O'Connor et al., 2008) and as high as 63% (Foley et al., 2012) of the MS population. The pain of MS is particularly problematic in primary-progressive and progressive-relapsing forms, seriously increasing disability (Nurmikko et al., 2010, Truini et al., 2011). As with other forms of neuropathic pain, the pain of MS is refractory to treatment. A typical study found that only 61% of patients reported pain relief, and of those, the extent of pain relief was less than 40% (Grau-Lopez et al., 2011). Here we review randomized controlled clinical drug trials in MS patients (uncontrolled studies are excluded) and experimental pharmacological studies in the experimental autoimmune encephalomyelitis (EAE) rodent model of MS.

2. Pathology of Multiple Sclerosis

MS is characterized by focal demyelinated areas (plaques) with variable shape, number and size in the white matter of the spinal cord and brain, as well as the grey matter in cerebral cortex, basal ganglia and thalamus (Haberland, 2007). While grey matter lesions are associated with neuronal pathology, white matter plaques are associated with axonal loss (Franklin et al., 2012). Microglial activation and astrogliosis occur in the early and chronic stages of disease respectively (Lassmann, 2010). Activated microglia phagocytose myelin debris and also inhibit oligogenesis (Bauer et al., 1994, Li et al., 2005, Zhang et al., 2011). Axonal injury, cortical demyelination, perivascular inflammation and neuronal changes have also been extensively described in MS (Hu and Lucchinetti, 2009, Vercellino et al., 2009).

Patterns of demyelinating lesions have been categorized by Lucchinetti et al (Lucchinetti et al., 2000): Pattern I demyelinating lesions are characterized by the presence of macrophages and T-cells within the lesions. Pattern II demyelinating lesions are characterized by the presence of macrophages and T-cells plus activated complement antigen (C9neo) and immunoglobulin (mainly IgG) deposition. Pattern III demyelinating lesions are characterized by the presence of macrophages, T-cells and activated microglia with damage to oligodendrocytes and significant loss of myelin-associated glycoprotein (MAG) but not other myelin proteins including proteolipid protein (PLP), myelin basic protein (MBP, and 2',3' cyclic nucleotide 3' phosphodiesterase (CNP). Finally, Pattern IV demyelinating lesions are characterized by the presence of T-cells and macrophages with damage to oligodendrocytes but no significant loss of MOG or other myelin components. Pattern I and II lesions have been detected in patients that present with all clinical subtypes of disease before biopsy or death. On the other hand, pattern III lesions have been observed mainly in

patients with a disease of less than 2 months duration before biopsy or autopsy. Pattern IV lesions have been observed in patients affected by primary progressive MS with prominent cerebellar and brainstem involvement and are associated with cognitive deficits (Lucchinetti et al., 2000).

3. Etiology of MS

MS is a multifactorial disease of unclear origin. A leading hypothetical cause of early MS lesions includes activation of myelin-specific T cells and macrophage/monocytes (Li et al., 1996, Huseby et al., 2012), damage to the endothelial blood brain barrier, and infiltration of T cells into the CNS (Goverman, 2009), leading to oedema formation and complement deposition (Wekerle, 2008). The contribution of T-cells to the pathogenesis of MS has been extensively reviewed (Anderton and Liblau, 2008, Miller, 2011). Microglial activation occurs in both demyelinating and inflammatory non-demyelinated regions of the brain throughout the course of disease (Wu and Tsirka, 2009, Mikita et al., 2011). Numerous other causes have also been proposed, including oedema, viral infection by Epstein-Barr virus (Pender et al., 2011), impaired cerebrospinal venous drainage (D'Haeseleer et al., 2011), and inhibition of oligogenesis by activated microglia (Bauer et al., 1994, Li et al., 2005, Zhang et al., 2011).

4. Diagnosis and chronic consequences of MS

Widely-accepted diagnostic criteria have been summarized by McDonald et al. (McDonald et al., 2001), and revised by Polman (Polman et al., 2005, Polman et al., 2011). Strong evidence of MS includes 2 or more “attacks” that involve clinical events characteristic of an acute CNS inflammatory demyelinating episode. These events last at least 24 hours and occur in the absence of fever or infection. Clinical events include spasticity, clumsiness of the hands, double vision (diplopia), temporary vision loss or blurred vision, numbness, pain, bladder, bowel and sexual dysfunction, impairment of coordination, and muscle weakness. A definitive diagnosis of MS includes corroboration of attacks with neurological examination and paraclinical laboratory assessments, namely magnetic resonance imaging (MRI), visual evoked potential responses (VEP), and cerebrospinal fluid (CSF) analysis. MRI quantifies the progression of white and grey matter lesions in the brain and spinal cord (Thorpe et al., 1996, Bastianello et al., 2000, Garcia-Lorenzo et al., 2012). Grey matter lesions can appear at early phases of disease, often correlating with development of cognitive disability (Calabrese et al., 2012). Evoked potentials aid the confirmation of clinically silent lesion sites (subclinical lesions). CSF is analysed for protein and glucose, lactate, myelin basic protein, CSF/serum albumin ratio, immunoglobulin-gamma (IgG), immunoglobulin-M (IgM), kappa light chains and oligoclonal IgG bands (Luque and Jaffe, 2007). Diagnostic criterion include positive brain MRI (9 lesions or 4 lesions with a positive VEP response), positive spinal cord MRI (two focal lesions), or positive CSF (Polman et al., 2011). The above criteria are concomitantly used to formulate a diagnosis; however, misdiagnosis of other diseases as MS remains a major problem (Singhal and Berger, 2012).

Consequences of MS, possibly associated with chronic pain, include spasticity, fatigue, depression, and cognitive impairment (Solaro and Uccelli, 2011). The pseudobulbar affect

(pathological laughing or crying) is present in 10% of the MS population (Smith et al., 2004)). The Expanded Disability Status Scale (EDSS) is widely used to monitor progression of MS-related symptoms (Iuliano et al., 2008), as it correlates strongly with laboratory diagnostic confirmations (Invernizzi et al., 2011).

Laser Evoked Potentials (LEPs) are cortical responses to a laser beam directed to the skin of the hand and foot and are often delayed or reduced in MS patients (Kakigi et al., 1992, Spiegel et al., 2003). LEPs reflect selective activation of A-delta and C nociceptors in the hairy skin (Treede et al., 2003, Cruccu and Garcia-Larrea, 2004), and indicate lesions or dysfunction of the spinothalamic tract (Spiegel et al., 2003). Somatosensory Evoked Potentials (SEPs) in response to stimulation of median or posterior tibial nerves may also be delayed in MS patients, and can indicate lesions or dysfunction of the dorsal column tract (Spiegel et al., 2003).

5. Clinical presentation of pain in MS

The International Association for Study of Pain (IASP®) recently introduced a revised definition of neuropathic pain as “pain caused by a lesion or disease of the somatosensory system” (Jensen et al., 2011). Although MS unequivocally involves CNS lesions of motor systems, it is not established whether lesions extend to the somatosensory system. Based on pain reports in MS patients, and use of LEPs to investigate the function of nociceptive fibres in patients affected by neuropathic pain (Cruccu et al., 2008, Haanpaa et al., 2011), we argue that neuropathic pain does exist in MS. For example, Truini et al found that, of 10 MS patients reporting ongoing extremity pain, 9 (90%) displayed LEP abnormalities, indicative of dysfunction in nociceptive pathways (Truini et al., 2012).

Pain severely impacts the quality of life of about half of the MS population, with up to 75% of patients reporting pain within the preceding month (O'Connor et al., 2008, Solaro and Uccelli, 2011, Foley et al., 2012). Up to a third of MS patients characterize pain as one of their worst symptoms, and analgesics represent a major class of medications used by them (Stenager et al., 1991, O'Connor et al., 2008, Solaro and Uccelli, 2011). Pain intensity can occur in newly or recently diagnosed cases, increases with disease severity (O'Connor et al., 2008, Truini et al., 2012), and varies widely from mild to severe across several studies. On average, most patients report moderate pain – approximately 5 on a 0–10 numerical rating scale. Pain in MS typically involves the musculoskeletal system (nociceptive pain) and/or the CNS (neuropathic pain), or both, and MS is associated with a high incidence of headache as well. The primary forms of neuropathic pain in MS are ongoing “dyesthetic” extremity pain, trigeminal neuralgia, and painful Lhermitte’s sign. These are frequently associated with painful muscle spasms and cutaneous allodynia / hyperalgesia (O'Connor et al., 2008). It is not uncommon for patients with MS to experience multiple types of pain.

Ongoing extremity pain is usually chronic, presenting as bilateral burning sensation at the legs and feet. It is a common form of pain in MS, being reported by almost 1 in 5 patients (O'Connor et al., 2008, Truini et al., 2011). It is particularly prevalent in primary progressive and progressive relapsing subtypes (O'Connor et al., 2008, Nurmikko et al., 2010).

Trigeminal neuralgia and optic neuritis (eye pain) present in approximately 5–20% of MS patients (Cruccu et al., 2009, Nurmikko, 2009, Truini et al., 2011). Compared to classic trigeminal neuralgia, MS-associated trigeminal neuralgia is more often bilateral in presentation (O'Connor et al., 2008). MS-associated trigeminal neuralgia is often associated with lesions of the intrapontine trigeminal primary afferents. Eye pain results from inflammation of the optic nerve trunk, thereby activating intraneural nociceptors innervated by nervi nervorum.

Lhermitte's sign (symptom) is evoked by neck flexion and presents as a sudden-onset, brief, electric shock-like sensation travelling rapidly down the spine, occasionally reaching the arms or leg (Kanchandani and Howe, 1982, Al-Araji and Oger, 2005, Nurmikko et al., 2010). It is frequently reported in MS patients with a prevalence of 9–41% (Solaro et al., 2004). Truini et al reported that of 18 MS patients with painful Lhermitte's, 13 (72%) exhibited abnormal SEPs (Truini et al., 2012), indicative of neuropathic dysfunction within the dorsal column/medial lemniscus system (Treede et al., 2003, Cruccu et al., 2008).

In addition to spontaneous pain, MS is often associated with cutaneous hypersensitivity, including allodynia and hyperalgesia. Many patients report heat hypersensitivity (58%), or paradoxical sensation of warmth elicited by a cold stimulus; such abnormalities likely contribute to fatigue, cognitive problems (*see Benson and Kerr in this volume*), and pain (Hansen et al., 1996, Morin et al., 2002, Flensner et al., 2011). Other patients present with cold allodynia (Osterberg et al., 2005, Svendsen et al., 2005), and some report tactile allodynia (Svendsen et al., 2004, Osterberg et al., 2005, Cruccu et al., 2009), namely brush-evoked allodynia (Sakai et al., 2004, Osterberg and Boivie, 2010).

Approximately 75% of MS patients present with a velocity-dependent increase in muscle reflexes (Rizzo et al., 2004), and muscle spasms are often associated with musculoskeletal pain. For example, approximately 10% of MS patients present with cramping and/or pulling descriptions of arm pain that are associated with tonic spasms (Solaro and Messmer Uccelli, 2010, Truini et al., 2011). Uncontrolled spasms can contribute to damage of descending motor pathways and motor neurons, leading to a vicious cycle of increased tone, gait abnormalities, fatigue and painful tonic spasms (Beard et al., 2003, Boissy and Cohen, 2007, Ben-Zacharia, 2011). On the other hand, in human subjects with CNS lesions, intrathecal baclofen suppressed dyesthetic and spasm-related pain with a different time course, suggesting that different mechanisms contribute to each pain type (Herman et al., 1992).

6. Clinical pharmacology of pain in MS

Pharmacological studies and treatments for MS typically target motor dysfunction, and often neglect neuropathic pain. For example, out of 134 patients reporting pain associated with MS, only 38% received treatment for their pain (42% NSAIDs, 27% anticonvulsants, 7% homeopathic, 24 % others (Grau-Lopez et al., 2011). This is due in large part to the fact that clinicians often fail to recognize the clinical features of pain, in part because they are difficult for patients to describe (Solaro and Uccelli, 2011). Furthermore, clinical trials in MS do not typically evaluate pain as an outcome measure. Of the clinical trials that have measured pain, the small cohorts of patients used, the open-label design of most studies, and

the lack of placebo controls does not allow for definitive conclusions to be drawn regarding analgesic efficacy of currently-prescribed analgesic drugs. Indeed, MS patients with neuropathic pain report low satisfaction with pain management (Solaro and Uccelli, 2011). Thus, we have a suboptimal situation where pharmacological management of neuropathic pain in MS is driven by anecdotal reports and by findings of clinical trials conducted in patients with very different forms of neuropathic pain, such as spinal cord injury or peripheral nerve injury (Nurmikko et al., 2010). Below and in Table 1, we summarize the results of blinded, randomized controlled trials (RCT) that have assessed pharmacological approaches to manage pain in MS. In general, open label studies are omitted from this review.

Cannabinoid receptor agonists

Sativex—Initial RCTs of this drug class assessed the analgesic efficacy of Sativex, an oromucosal spray of Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD). This preparation was thought to antagonize some THC-induced adverse events such as intoxication, sedation and tachycardia, while contributing its own analgesic and anti-emetic properties (Russo and Guy, 2006). Conflicting results have been reported in double-blind RCTs using Sativex. While two studies showed no reduction in MS-pain using Sativex (Wade et al., 2004, Conte et al., 2009), several others have yielded promising results. First, a small RCT reported that Sativex decreased VAS by greater than 50% in 16 out of 34 MS patients, but also produced several side effects (Notcutt et al., 2004). Similarly, Rog and colleagues reported that Sativex reduced the intensity of neuropathic pain (Rog et al., 2005), although again, dizziness and nausea were reported as common side effects in a follow-up study (Rog et al., 2007). During the randomized-withdrawal phase, Sativex was still significantly effective (Langford et al., 2013). Open label follow-up studies report that Sativex-responsive patients continue to receive pain relief for over a year (Wade et al., 2006, Rog et al., 2007). A more recent placebo-controlled study found that a 10-wk regimen of Sativex significantly decreased pain numerical rating scale on a 10-point numerical rating scale (improvement equal or superior to 30 %) (Langford et al., 2013).

Other cannabinoid preparations—Oral cannabis extract was assessed in 211 MS patients and THC was assessed in a similar sized cohort of 206 MS patients. Both treatments significantly reduced pain scores as assessed by category rating scale (Zajicek et al., 2003). Furthermore, synthetic THC, dronabinol, significantly reduced the median spontaneous pain intensity and radiating pain when compared to placebo (Svendsen et al., 2004). Nabilone, a synthetic cannabinoid, has also been found to reduce the pain associated with spasticity, without serious adverse events (Wissel et al., 2006). Cannabis preparations that contained THC and cannabidiol improved self-reported pain assessed by an eleven point category-rating scale in a randomized placebo-controlled clinical trial. At 4 and 8 weeks, body pain was significantly decreased and, at 12 weeks, the proportion of relief in patients receiving cannabis extract was nearly double that reported by subjects receiving placebo (Zajicek et al., 2012). Furthermore a randomized, double-blind clinical trial of smoked cannabis showed a significant improvement in VAS pain (Corey-Bloom et al., 2012). Thus, in contrast to the mixed effects of Sativex, administration of other cannabinoid receptor agonist preparations typically reduce the pain of MS. In summary, cannabinoid receptor agonists hold

considerable promise for the treatment of MS pain. However, these compounds are often associated with a significant amount of side effects including dizziness, headache, fatigue and impaired judgement, and so have been restricted to 2nd or 3rd-line treatments for neuropathic pain, or to adjunctive treatments as in the case of Sativex (Attal et al., 2010, Dworkin et al., 2010).

Despite an enormous amount of interest in cannabinoids for the neuropathic pain of MS, all clinical trials to date have been limited by a low number of patients. Studies are also limited by the selection of patients with just the secondary-progressive subtype of MS, or absence of discrimination between MS subtypes.

Opioids

Despite the widespread use of opioids for chronic pain, remarkably few studies have investigated their efficacy for the neuropathic pain of MS. A single dose using intravenous morphine decreased MS pain (>50% pain reduction) in 29% of the 14 patients involved in a single-blind non-randomized placebo-controlled trial (Kalman et al., 2002). Opioid receptors appear to mediate the analgesic effect of morphine, as it was reversed by naloxone. This reduction in pain was visible only at high doses of morphine (mean = 41 mg), indicating weak efficacy. Thus, strong opioid analgesics have been relegated to 2nd line treatment for the pain of MS (Dworkin et al., 2010).

Anticonvulsants

Anticonvulsants are a first-line treatment for several forms of neuropathic pain, including trigeminal neuralgia, postherpetic neuralgia and painful diabetic neuropathy (Jensen, 2002). As a result, they are also prescribed for the pain of MS, particularly carbamazepine for MS-related trigeminal neuralgia. However, the small number of RCTs conducted so far do not demonstrate substantial pain relief or tolerability in MS patients. Levetiracetam (500 mg) was shown to significantly reduce VAS scores in MS patients with pain in a randomized double-blind placebo-controlled clinical trial (Rossi et al., 2009). In this study, degree of pain reduction was correlated to the severity of pain at baseline ($r^2 = 0.55$, $P < 0.05$). Higher doses of Levetiracetam (3000 mg) also reduced pain on a 10-point numeric scale in a randomized double-blind placebo-controlled clinical trial (Falah et al., 2011). However, this effect was limited to a subgroup of patients without touch-evoked or lancinating pain. By contrast, a well-done pilot study found that the anticonvulsant lamotrigine had no efficacy for pain in MS, and the authors concluded that larger scale studies were not needed (Breuer et al., 2007). Oxcarbazepine, a keto-analogue of carbamazepine, was found to reduce painful paroxysmal symptoms of MS in an open-label pilot study (Solaro et al., 2007). Gabapentin, a commonly prescribed drug for neuropathic pain also reduced MS-related pain in an open-label study, but several of the patients experienced dose-dependent side effects including mental cloudiness, confusion, dizziness and irritability (Houtchens et al., 1997).

Tricyclic antidepressants (TCA)

Despite sparse clinical trials, TCAs are recommended as first-line medicines for the management of the pain of MS (Solaro and Uccelli, 2011, Truini et al., 2011). One randomized trial compared nortriptyline with self-applied transcutaneous electrical nerve

stimulation (TENS) rather than placebo (Chitsaz et al., 2009). Nortriptyline was as effective as TENS in reducing VAS scores, but produced substantially more side effects. A promising Phase 3 RCT found that duloxetine reduced 24-hour pain scores on an 11-point Likert scale as compared to placebo (Eli Lilly & Co)

Muscle relaxants

Intrathecal administration of the γ -aminobutyric acid class B (GABA_B) receptor agonist baclofen is used for the treatment of spasticity, and so was tested in patients with MS. In a double-blind RCT, baclofen decreased dysesthetic pain (continual, spontaneous burning, lancinating, shooting, radiating knife-like feelings), as well as spasm-related pain and pinch-induced pain (Herman et al., 1992). Baclofen, or newly available GABA_B receptor agonists such as arbaclofen, could be interesting candidates for future RCTs for MS pain.

7. Animal models of multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE)

EAE models in mice and rats remain the most widely used translational tools in multiple sclerosis research (Baxter, 2007). Although important differences between EAE and MS pathologies have been pointed out (Croxford et al., 2011), there are many similarities including clinical course, pathological CNS lesions, glial activation, and axonal demyelination (Mix et al., 2010, Handel et al., 2011). For example, EAE rodents exhibit widespread demyelination, anti-MOG antibodies, and MOG-reactive T-cells, similar to the CNS of patients with MS (Storch et al., 1998, Iglesias et al., 2001). Thus, EAE has provided fundamental advances in our understanding of MS pathology (Linker and Lee, 2009) and in the development of new pharmaceutical therapies (Kieseier and Hartung, 2003, Steinman and Zamvil, 2006). Most EAE models involve the systemic administration of encephalitogenic myelin antigens to a range of species including mice, rats, guinea pigs and monkeys. Bacterial components such as heat inactivated mycobacteria are added to an adjuvant (Freund's adjuvant) to activate the innate immune system and to increase sensitization to myelin antigens (Gold et al., 2006, Constantinescu et al., 2011). Pertussis toxin (PTx) is co-injected as it elicits a type 1 differentiation of T cells and opens the blood brain barrier (BBB), facilitating the migration of pathogenic T cells into the CNS (Hofstetter et al., 2002, Darabi et al., 2004). Histopathology and clinical course of the disease varies considerably with the type and dose of antigens and adjuvants, and species (Lassmann, 1983, Khan and Smith, 2013).

EAE using MOG

Myelin oligodendrocyte glycoprotein (MOG) is a component of the outer myelin layer. MOG is the most commonly used antigen to model MS because its expression is restricted to the CNS, and because it produces clinical symptoms similar to MS that generally are stable or gradually worsen – they do not resolve over time. Subcutaneous injection of a fragment of MOG peptide (MOG_{35–55}), induces encephalitogenic T cells, demyelination, axonal loss, and clinical signs of neuromuscular dysfunction in C57BL/6 and Biozzi ABH mice (Linker and Lee, 2009). Patterns of EAE-induced pathology vary considerably with the dose and frequency of antigen administration. For example, a single injection of low dose

MOG_{33–55} (50 µg) and PTx (200 ng) produces a mild monophasic form of relapsing remitting EAE in which remission is not followed by subsequent symptoms. By contrast, higher doses of MOG_{33–55} (300 µg) and PTx (300 ng) produce a more chronic form of EAE characterized by larger inflammatory lesions, myelin loss, axonal damage and clinical signs that worsen with time (Berard et al., 2010). Each of these models has been used to study different forms of MS. Recent work, however, suggests that changes in pain sensitivity are a common feature of MOG_{35–55} induced EAE regardless of the source of the MOG or the concentration of adjuvant used to induce the disease (Olechowski et al., 2009, Olechowski et al., 2013).

Other EAE models

Other commonly-used antigens include proteolipid protein (PLP) and myelin basic protein (MBP). These are not as commonly used as MOG because they are expressed not only in the CNS, but also in the peripheral nervous system. PLP causes CNS inflammation, neuritis and radiculitis in the absence of frank demyelination (Pender, 1988). MP4 is an MBP-PLP fusion protein that causes demyelination at the onset of clinical symptoms (Kuerten et al., 2011). A severe form of EAE results from the daily administration of cyclosporine for 21 days after MOG-induced EAE (Thibault et al., 2011). A model of chronic-progressive MS can be generated by intracerebroventricular inoculation with Theiler's murine encephalomyelitis virus (TMEV). TMEV inoculation leads to spinal cord and brain inflammation, demyelination and axonal damage (Fuller et al., 2004, Oleszak et al., 2004, Tsunoda and Fujinami, 2010). The TMEV model supports the hypothesis that the etiology of MS includes viral infection (Olson et al., 2005).

8. Behavioral signs of neuropathic pain in EAE

An emerging body of work demonstrates that pain-like cutaneous hypersensitivity at the tail and hindpaws is a reliable symptom associated with EAE models, and mimics the lower extremity pain reported by MS patients (Svendsen et al., 2005, O'Connor et al., 2008, Nurmikko et al., 2010, Truini et al., 2011). The following sections summarize the characteristics of these behaviors, describe part of the neuropathology underlying them, and then illustrate the limited preclinical studies to date to generate new targets for pain relief in MS patients.

Tactile hypersensitivity

Mechanical allodynia, as assessed with calibrated von Frey hair monofilaments applied to the dorsal or plantar surface of the hind paws, has been found in several EAE models. Compared to vehicle treated controls (mice immunized with only the CFA adjuvant), MOG_{35–55} consistently increases tactile sensitivity. For example, compared to complete Freund's adjuvant (CFA) treated controls, MOG decreased response threshold (Olechowski et al., 2009, Rodrigues et al., 2009). Mechanical allodynia has also been described in a rat model of chronic MOG_{1–125}-induced EAE (Ramos et al., 2010). On the other hand, using higher doses of MOG_{35–55} to induce more severe clinical symptoms, mechanical allodynia may not manifest (Lu et al., 2012). The reasons for this are unclear, but it is quite possible that the motor deficits associated with higher doses of MOG are severe enough to interfere

with the observation of pain behaviors. Indeed, as further discussed below, the interpretation of pain behaviors and dorsal horn neurophysiology in EAE models requires a careful consideration of motor and reflex function at the ventral horn and brain.

Cold hypersensitivity

As observed in MS patients, rodents with EAE exhibit increased sensitivity to cold stimuli (Morin et al., 2002 check refs, Osterberg et al., 2005, Svendsen et al., 2005). Compared to vehicle (CFA) controls, MOG_{35–55} consistently increased the response duration to innocuous cold stimuli delivered to the ventral surface of the hindpaw (Olechowski et al., 2009). Cold hyperalgesia at the tail or paw have also been observed in MBP-induced EAE models as assessed by the cold plate test (Thibault et al., 2011).

Heat hypersensitivity

As observed in MS patients (Flensner et al., 2011), rodent EAE models exhibited heat hypersensitivity. For example, PLP_{139–151}-induced EAE in SJL/J mice led to a heat hypersensitivity in the tail of male or female mice (Aicher et al., 2004). Both PLP_{139–151}-induced relapsing-remitting EAE in SJL mice and MOG_{33–55}-induced chronic EAE in C57BL/6 mice exhibited heat hypersensitivity during the chronic phase (day 35 to 45 after EAE induction) (Lu et al., 2012). TMEV infection also induced heat hypersensitivity in male and female SJL/J mice, as compared to control non-infected mice in the thermal tail-immersion test (Lynch et al., 2008). In a rat MBP model of EAE, heat hypersensitivity developed at the tail (Thibault et al., 2011). An exception was reported by Olechowki et al, who found no difference in hindpaw heat withdrawal thresholds of mice treated with MOG_{35–55} (Olechowski et al., 2009).

Relative time course of EAE pain and motor dysfunction

MOG_{35–55} produces mechanical hypersensitivity in mice within 7 days of immunization, well before the onset of frank neuromuscular deficits (Olechowski et al., 2009, Rodrigues et al., 2009, Yuan et al., 2012). Similarly, mechanical allodynia in male and female SJL/J mice occurs before or during the onset of motor deficits in the TMEV model (Lynch et al., 2008). Also, heat and cold hypersensitivity in rat MBP EAE models begins before the onset of motor dysfunction, and heat hyperalgesia continued even after clinical signs had disappeared (Thibault et al., 2011). However, due to differences in various parameters of the EAE model, a smaller number of studies indicate that thermal and mechanical hypersensitivity can develop after the onset of motor dysfunction in severe mouse MOG and PLP and rat MBP models of EAE (Thibault et al., 2011, Lu et al., 2012). Furthermore, a clinical study reported that MS patients present with sensory and motor symptoms of MS a year before complaints of pain (Osterberg et al., 2005). Thus, the majority of studies in animal models indicate that pain hypersensitivity typically precedes motor dysfunction in EAE models.

Sex differences in EAE pain

Despite the large difference in the prevalence of MS in women as compared to men, only a small number of rodent studies have compared EAE pain in both sexes. In two studies, thermal hyperalgesia after PLP_{131–151} or TMV was similar between male and female mice

(Aicher et al., 2004, Lynch et al., 2008). By contrast, TMEV mice develop mechanical allodynia faster in females than in males, despite the more rapid disease progression in males (Lynch et al., 2008). These findings support the idea, discussed below, that neuromuscular deficits do not prevent the study of pain hypersensitivity in EAE.

9. Neuropathological mechanisms of neuropathic pain in EAE-MOG models

EAE models are associated with damage to spinal and supraspinal pain transmission pathways. MOG_{33–55} rapidly (within 7 d of administration) and dramatically (>50%) damages the fasciculus gracilis of the medial dorsal column, with no significant remyelination at 1 year (Jones et al., 2008). This can lead to sensory deficits, since the dorsal columns normally carry non-noxious tactile information from large myelinated primary afferent neurons to the dorsal column nuclei. However, under certain pathological conditions, this information may be perceived as painful (Ossipov et al., 2002). Pain inhibitory GABA_B receptors are expressed at high concentrations in the superficial dorsal horn (Price et al., 1987, Margeta-Mitrovic et al., 1999, Castro et al., 2004), and clinical studies in patients with CNS lesions indicate that spinal GABA_B dysfunction may contribute to central pain, including MS pain (Herman et al., 1992).

EAE models are also associated with early changes in the reactivity of glial cells residing within the superficial dorsal horn. Activation of microglia and astrocytes correlates with the emergence of aberrant pain behaviours. For example, the mechanical and cold allodynia following MOG_{35–55}-induced EAE was associated with an increase in glial fibrillary acidic protein (GFAP) immunoreactivity and the number of CD3⁺ T-cells in the superficial dorsal horn of the spinal cord. GFAP and CD3⁺ T-cells were significantly increased not only during the onset and peak phases of EAE, but also during the chronic phase of stable motor dysfunction, 28 days post-MOG. MOG also increased F4/80 expression (labelling of microglia/macrophage) in the dorsal horn, particularly during the chronic phase (Olechowski et al., 2009). Similarly, in a model of relapsing-remitting EAE in SJL mice, the number of GFAP- and Iba1-immunoreactive cells in spinal cord dorsal horn was increased at the onset, peak and chronic phases of disease (Lu et al., 2012). Rat models of chronic and relapsing-remitting EAE also are associated with increased GFAP and CD11b immunoreactivity in the dorsal horn (Ramos et al., 2010). Thus, activated astrocytes and microglia may contribute to neuropathic pain in MS, just as they do in neuropathic pain models involving traumatic nerve injury.

For an excellent, more extensive review of additional pathobiology of MS-neuropathic pain in EAE models, see Khan and Smith (Khan and Smith, 2013).

11. Pharmacological studies in animal models of MS pain

Despite the prevalence of neuropathic pain in MS and the need for new treatments, pharmacological studies in experimental models are remarkably sparse. As noted above, several small clinical trials have investigated the effects of cannabinoid receptor agonists and anticonvulsants in MS-related pain, and single controlled studies have evaluated other targets. Only a few EAE studies, however, have been conducted to validate these approaches with currently available drugs, or to investigate new drugs for the future pharmacotherapy of

central neuropathic pain. Below we outline the results of these pharmacology studies, most of which are limited to a single dose of a single drug class. Surprisingly, none have reported on the effects of cannabinoid receptor agonists on EAE pain. For a more comprehensive list of potentially “druggable” targets in MS-associated pain, see Kahn and Smith (Kahn and Smith, 2013).

Anticonvulsants

Gabapentin is a first line therapy for the neuropathic pain associated with painful diabetic neuropathy and post herpetic neuralgia. In a rat EAE model, two weeks of daily gabapentin administration reduced mechanical hyperalgesia but not neuromuscular dysfunction (Thibault et al., 2011). Although only a single dosing regimen was used, the results of this study support the early clinical studies assessing the efficacy of anticonvulsants for pain in human EAE.

Serotonin-norepinephrine reuptake inhibitors (SNRIs)

Both tramadol and duloxetine inhibit the uptake of serotonin and norepinephrine in neuronal terminals, and this action is thought to contribute to their efficacy in reducing clinical neuropathic pain. Both of these drugs prevented the development of cold allodynia at 21 and 35 days after induction of EAE in rats (Thibault et al., 2011). Like gabapentin, neither tramadol nor duloxetine reduced motor dysfunction. Although only a single dosing regimen was used, this study points to the need for further clinical studies assessing the efficacy of SNRIs in human EAE.

mTOR inhibitors

Cytokine-driven T cell proliferation likely contributes to the induction of MS, as well as to the induction of motor dysfunction in EAE models (Huseby et al., 2012). Repeated administration of the immunosuppressant and mTOR inhibitor, rapamycin (Mondino and Mueller, 2007), begun 2 days after induction of EAE, significantly reduced mechanical allodynia at a single time point 2 weeks later, and produced a more complete prevention of motor impairment (Lisi et al., 2012). Additional studies are needed to determine whether rapamycin inhibits neuropathic pain in EAE by reductions in T cell activity and proliferation coincident with reductions motor impairments, or by a distinct mTOR activation mechanism within spinal pain transmission pathways (Geranton et al., 2009, Asante et al., 2010, Norsted Gregory et al., 2010).

Inhibitors of glutamatergic neurotransmission

Glutamatergic neurotransmission within spinal pain transmission pathways or at sclerotic lesion sites may contribute to the pain of EAE and MS. Neuropathological data indicate that EAE animals exhibited decreased excitatory amino acid transporter 2 and increased glutamate type 1 transporter (Olechowski et al., 2010, Ramos et al., 2010) and MS patients exhibit greater concentrations of excitatory amino acids in cerebrospinal fluid (Sarchielli et al., 2003), glutamate receptor expression on oligodendrocytes at the borders of active lesion sites (Newcombe et al., 2008), and other signs of altered glutamate homeostasis (Werner et al., 2001). An emerging pharmacology is supporting the idea that inhibition of glutamatergic

signaling decreases neuropathic pain in EAE. For example, ceftriaxone is not only a third-generation cephalosporin antibiotic, but also upregulates the spinal expression of excitatory amino acid transporters (EAAT), thus reducing synaptic glutamatergic neurotransmission. In both mouse and rat MOG models of EAE, daily intrathecal injection of ceftriaxone prevented behavioral signs of neuropathic pain (Ramos et al., 2010, Olechowski et al., 2013). In the rat, ceftriaxone also slowed the progression of paralysis and reduced molecular signs of astrocyte and microglial activation, which may contribute to sensitization of spinal pain transmission (Ramos et al., 2010).

12. Conclusions

Although ignored for many years, pain in MS is now recognized as an important feature of the disease that significantly impacts quality of life in roughly half of MS patients. Up to a third of MS patients characterize pain as one of their worst symptoms (Stenager et al., 1991, 1995). Sclerotic lesions in MS likely impinge on the somatosensory system, and therefore MS pain associated with ongoing extremities pain, trigeminal neuralgia, and L'hermitte's sign can be classified as neuropathic. Other forms of MS-associated pain may arise from peripheral somatosensory dysfunction or a mixed nociceptive, neuropathic pain (Khan and Smith, 2013).

Of the handful of clinical studies that have addressed pain in MS, most are severely limited by an open-label design. Of the very small subset that were designed with proper randomization, placebo control, and blinding, insufficient numbers of subjects prevents any firm conclusion. Clinical studies with anticonvulsants are promising, and studies with cannabinoid-based drugs suggest the CB1 and CB2 cannabinoid receptors to be quite a compelling target for future studies.

Conventional EAE mouse models, particularly those involving administration of MOG as the antigen, yield robust tactile and thermal hypersensitivities that mimic evoked pain in MS patients. MOG-based models are particularly attractive because MOG expression is restricted to the CNS, clinical symptoms are stable or increase over a long time period, and neuropathic pain-like behaviors are robust. Importantly, the majority of studies in EAE models indicate that behavioral manifestations of pain hypersensitivity typically precede motor dysfunction. We suggest that neuroplasticity of nociceptive transmission in the dorsal horn develops quickly in EAE as in other animal models of neuropathic pain, while relatively more time is required for the accrual of adequate demyelination and/or axonal injury before the behavioral manifestation of motor impairments. The practical ramifications of this temporal dissociation are that EAE-induced motor impairment does not prevent the observation and study of pain hypersensitivity, particularly in the earlier course of the disease. Future studies will likely reveal specific neuroplastic mechanisms within nociceptive pathways of the dorsal horn that contribute exclusively to the behavioral manifestations of chronic neuropathic pain.

Such neuropathological mechanisms of EAE pain are just beginning to be understood, and could involve dysfunction of glia or glutamatergic systems within the spinal cord dorsal horn. MOG-based EAE models have yielded some promising candidates from clinically-

available drugs including anticonvulsants, SNRIs, and mTOR inhibitors, and these studies provide proof-of-principle that studies in EAE hold great promise for the development of a new pharmacotherapy for the neuropathic pain associated with MS.

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Table 1

Clinical pharmacology of chronic pain in MS: randomized, placebo-controlled, blinded clinical trials. This table only lists clinical trials that were randomized and controlled, containing 12 or more subjects, and for which multiple reports within a particular drug class are available.

Reference	Treatment	#subjects	Pain Scale	Outcome	Pain as the primary endpoint?
Cannabinoid-based drugs					
Zajicek et al., 2003	Cannabis extract or THC.	611	CRS	Reduced patient-reported pain	No
Notcutt et al., 2004	Sativex®	34	VAS	Decrease VAS, p<0.05 Decrease>50% in 16/34 patients	No
Svendsen et al., 2004	Marinol, 3 wks	24	NRS	Decreased NRS, increased pain relief, increased SF-36	No
Wade et al., 2004	Sativex®	160	VAS	No change, p>0.05	Yes*
Rog et al., 2005	Sativex®	64	NRS	Sativex® reduced mean intensity of pain (p = 0.005).	No
Conte et al., 2008	Sativex®	17	RIII reflex; VAS	Decrease in RIII reflex. No change in VAS	Yes
Corey-Bloom et al., 2012	Cannabis cigarettes (4% THC)	30	VAS	VAS pain reduced by an average of 5.28 points vs. placebo	No
Zajicek et al., 2012	Cannabis extract, 2.5–25 mg THC	279	NRS	self-reported relief from body pain at 4, 8 and 12 weeks	No
Langford et al., 2013	Sativex®	339	NRS	Significantly reduced pain by week 10; otherwise, no effect	Yes
Anticonvulsants					
Breuer et al. 2007	lamotrigine	12	BPI, NPS, MSQOL-54	No effect of drug	Yes
Rossi et al., 2009	Levetiracetam	20	VAS	Significant VAS pain reduction (P < 0.05)	Yes
Falah et al., 2011	Levetiracetam	27	6-point verbal scale pain relief	No effect except in patients with lancinating or without touch-evoked pain	Yes

Abbreviations: VAS: Visual Analogue Scale; NRS: Numerical Rating Scale; CRS: Category rating scale for self-reported pain; BPI: Brief Pain Inventory; NPS: Neuropathic Pain Scale; MSQOL: Multiple Sclerosis Quality of Life Inventory; SF-36: SF-36 quality of life scale; THC: 9-tetrahydrocannabinol.

* pain as the primary endpoint only in a subset of 36 patients.