Voltage-gated potassium channels in multiple sclerosis: Overview and new implications for treatment of central nervous system inflammation and degeneration

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Abstract—Inflammatory tissue damage and the presence of reactive immunocompetent T lymphocytes, macrophages, microglia, and dendritic cells (DCs) are characteristic features in the human chronic inflammatory demyelinating disease, multiple sclerosis (MS). Together, these cells orchestrate the inflammation and immunopathogenesis underlying the MS autoimmune disease processes and all up-regulate the same voltage-gated potassium (Kv) channel, Kv1.3, when fully activated. Only microglia, which mediate central nervous system (CNS) inflammatory processes (possibly playing a dual role of CNS protection and mediation of neuroinflammation/neurodegeneration), and DC, which are pivotal to the induction of T cell responses, express the distinct Kv1.5 prior to Kv1.3 up-regulation. Although the precise functional roles of first Kv1.5 and then Kv1.3 channels are unclear, their differential expression is likely a common mechanism used by both microglia and DC, revealing Kv1.5 (in addition to Kv1.3) as a potentially important target for the development of new immunomodulatory therapies in MS.

INTRODUCTION

Multiple sclerosis (MS) is a chronic and progressive neurodegenerative disease for which no cure exists. Considered a primary inflammatory disease of central nervous system (CNS) white matter, pathological lesions in MS are characterized by inflammatory demyelination with relative sparing of axons [1], perivascular/parenchymal infiltration of T lymphocytes (T cells) and macrophages.

Abbreviations: 3,4-DAP = 3,4 diaminopyridine, 4-AP = 4-aminopyridine, BBB = blood-brain barrier, CD = clusters of differentiation, CNS = central nervous system, CSF = cerebrospinal fluid, DC = dendritic cell, EAE = experimental allergic encephalomyelitis, EAN = experimental allergic neuritis, IL = interleukin, Kir = inward rectifying potassium (channel), Kv = voltage-gated potassium channel, MBP = myelin basic protein, MHC II = major histocompatibility class II, MS = multiple sclerosis, MuLV = murine leukemia virus, NADPH = nicotinamide adenosine dinucleotide phosphate, NO = nitric oxide, NOS = NO synthase, PNS = peripheral nervous system, T cells = T lymphocytes.

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[1–3], and proliferation and activation of resident microglia and astrocytes [4], as well as peripheral dendritic cells (DCs) [5]. In addition to inflammation and demyelination (white and gray matter), axonal damage and loss are now recognized as contributing to irreversible deficits in MS [6]. Clinical symptoms include blurred vision, unstable balance, poor coordination, tremors, numbness, and slurred speech, for which the underlying physiological impairment is believed to be conduction block arising from demyelination and inflammation.

Current approaches to treating MS patients include symptomatic treatment of neurological deficits and immunomodulatory therapy to treat neuroinflammation and possibly limit neurodegeneration. Voltage-gated potassium (Kv) channels are potential targets for both types of therapies. As symptomatic therapies, only two relatively nonspecific blockers of Kv channels, 4-amino-pyridine (4-AP) and 3,4 diaminopyridine (3,4-DAP), have been tested clinically for their efficacy in the treatment of patients with MS [7–15]. To date, in vivo immunosuppressive treatments that use nonspecific (4-AP and quinidine) and various highly selective Kv channel blockers (margatoxin, correolide, kaliotoxin, ShK, and Sh-Dap22) have been restricted to miniswine [16–17] and rodent experimental allergic encephalomyelitis (EAE) [18–20] animal models for MS.

The first study implicating a Kv blocker (quinidine) as a successful therapeutic treatment in an inflammatory demyelinating disease was an animal model performed in rats with experimental allergic neuritis (EAN), an accepted animal model for the human Guillain-Barre syndrome that is the peripheral nervous system (PNS) counterpart of EAE in the CNS. Mix and colleagues demonstrated that injecting EAN rats with quinidine ameliorated symptoms of clinical EAN [21]. These neurological benefits were accompanied with reduced inflammatory infiltrates in target tissue but not improved peripheral nerve conduction, thus foreshadowing the emerging view that Kv blockers may primarily exert their neurological benefits in MS through immunomodulatory effects.

**TARGETING Kv CHANNELS AS SYMPTOMATIC TREATMENT IN MULTIPLE SCLEROSIS**

The original clinical rationale for using Kv channel blockers to improve neurological function in the symptomatic treatment of patients with MS stemmed from physiological demonstrations in the PNS in which blocking paranodal or intermodal Kv channels prolonged action potential and potentiated synaptic transmission [22–25]. Many intact nonconducting axons in MS lesions can but do not conduct because their safety factor for conduction is fractionally below unity [26]. The recruitment of such axons by simply reducing body temperature [27] or changing serum-ionized calcium [28] raised hope that many more axons could be recruited pharmacologically with the use of Kv channel blockers. Waxman gives a current review of underlying disease processes and neuronal injury in MS [29]. Judge and Bever provide a current review of Kv channels as symptomatic targets in MS [30].

Although clearly beneficial, both 4-AP and 3,4-DAP are potent convulsants with narrow therapeutic windows that have limited their widespread clinical use in MS treatments. Toxic, epileptogenic side effects likely arise from the indiscriminate blockade of widely distributed and varied CNS Kv channels rather than Kv channels along demyelinated nerve fibers. Initially, the clinical improvements achieved in MS patients with 4-AP primarily were viewed as likely arising from blockage of Kv channels exposed on demyelinated nodes. In an experimental in vitro CNS study, Perreault and Avoli showed that seizure induction by 4-AP results from block of a synaptic channel [31]. More recently, Smith et al. undertook the first and only in vivo CNS studies in rats of 4-AP on experimental demyelination [26]. Their studies indicated that clinical doses of 4-AP probably produced beneficial neurological effects, not by blocking Kv channels in demyelinated axons, but by blocking Kv channels that promote synaptic transmission and increase skeletal muscle twitch tension, independent of demyelination. Understanding the clinical/therapeutic effects of 4-AP is complicated: (1) 4-AP blocks a wide variety of Kv channels that are distributed across multiple cell types in the CNS (neurons and microglia) and in the immune system (T cells, macrophages, and DCs) and (2) the molecular identities of the Kv channels actually targeted by 4-AP, clinically, remain unknown.

**Kv CHANNELS IN IMMUNE CELLS INTEGRAL TO MULTIPLE SCLEROSIS**

Together, reactive immunocompetent T cells, macrophages, microglia, and DCs orchestrate the inflammation and immunopathogenesis underlying MS autoimmune
disease processes. Each immune cell type is characterized by a cell-specific repertoire of ionic channels, a state-specific/differential expression of distinct Kv channels (Figure 1).

Macrophages are involved in chemotaxis, active myelin breakdown, phagocytosis of myelin proteins, myelin antigen presentation, and cytokine secretion. Microglia mediate proinflammatory immune responses, generate nitric oxide (NO)/elevated NO synthase (NOS) in MS lesions, and are active in myelin breakdown, phagocytosis of myelin proteins, and myelin antigen presentation. DCs initiate and regulate T cell responses and may contribute to inflammation relapses and chronicity and breakdown of tolerance to autoantigens.

Activated, immunocompetent T cells [18–19,32–37], macrophages [38–39], microglia [40–46], and DCs [47] up-regulate the same Kv channel, Kv1.3. Resting DCs [48], macrophages [49–54], and microglia [41,55–56] transiently exhibit an inwardly rectifying Kv channel (Kir). However, only DCs [48,57] and microglia [58] express the distinct Kv1.5 channel in addition to Kv1.3 following stimulation; microglia express Kv1.5 between their unstimulated and fully activated states, but DCs express a mix with Kv1.5 predominant. While murine bone marrow-derived macrophages have been shown to express Kv1.5 messenger ribonucleic acid [39], to date, no Kv1.5 currents have been recorded in macrophages. Figure 2 outlines functional roles of various potassium channels in T cells, macrophages, microglia, and DCs.

Apart from the known central role of cell-mediated immune responses in MS, accumulating evidence indicates that humoral immune responses (i.e., effector B lymphocytes) may also contribute to the pathogenesis of MS. Such evidence includes the identification of antimyelin antibodies in MS lesions, serum and cerebrospinal fluid (CSF) [59–63], and clinical observations consistent with antibody-mediated demyelination in an MS patient [64]. Furthermore, serum antimyelin antibodies in patients initially presenting with a clinically isolated syndrome may predict early conversion to clinically definite MS [65–66]. While the clinical and pathological significance of antimyelin antibodies in MS remains to be definitively characterized, activated B cells, like T cells, macrophages, microglia, and DCs, also up-regulate Kv1.3 channels that are already recognized as putative therapeutic targets in MS. First recorded in B cells by Choquet and Korn [67–68], Kv1.3 currents have been shown to be functionally important [69–75], indicating that B cells may constitute yet another immune cell target for putative immunomodulatory therapies designed to act via effects on Kv1.3 channels.

TARGETING Kv CHANNELS AS IMMUNOSUPPRESSIVE THERAPY

While the toxic, epileptogenic side effects resulting from 4-AP likely arise from the indiscriminate blockade...
of various CNS Kv channels, blockade of 4-AP-sensitive Kv channels in immune cells has emerged as a promising candidate because of its neurological benefits. Beneficial 4-AP effects could arise not only from blockade of CNS synaptic channels [26] but also from effects on microglia [43,58] and/or T cells [33–34]. Notably, the Kv1.3 is the predominant Kv channel in both activated T cells [34,76] and activated microglia [58]. The identification of Kv1.3 in mature antigen-presenting DCs [47] implicates these cells as an additional likely candidate contributing to the beneficial neurological effects of 4-AP or 3,4-DAP treatment in MS patients. Recently, high Kv1.3 expression was demonstrated in the perivenular and parenchymal inflammatory infiltrates in postmortem MS brain, as well as on CSF T cells from MS patients [77].

T Cells

Studies of Kv1.3 in activated T cells predate the cloning of Kv channels. Dating back to the mid-1980s, the first recordings in human peripheral blood T cells showed inhibition of mitogen-stimulated activation by nonspecific Kv channel blockers [32–33,37]. This finding was followed by the first studies in myelin basic protein (MBP)-reactive rat T cells [35–36,78–80] and the first demonstration that 4-AP and other nonspecific Kv channel blockers (e.g., tetraethylammonium, methoxyverapamil) could inhibit the adoptive transfer of relapsing-remitting EAE in rats [35–36,80]. More recent studies have determined the molecular identities of T cell Kv channels and shown differential expression of these channels in response to acute versus
chronic MBP stimulation [18–19,81]. Considerable advances have been made in identifying potent toxins [34,79,82–83] that are highly selective blockers for the T cell Kv1.3 channel, with better selectivity/potency profiles and experimental therapeutic effects [18–19] than 4-AP [35–36,78,80]. While proving successful, systemic administration of highly selective Kv1.3 blocking agents in EAE still has not shown whether it produces beneficial neurological effects by blocking Kv1.3 in T cells, microglia, macrophages, neurons, and/or DCs.

**Dendritic Cells**

DCs are a major component of the innate immune system and play a pivotal role in the adaptive immune response by providing necessary costimulatory signals for the induction of T cell responses, surface-expressed complexes of antigen peptide, and major histocompatibility class II (MHC II) molecules. Immature DCs are proficient at antigen endocytosis and processing but poor at stimulating T cells. Terminally mature DCs are proficient antigen-presenting cells highly specialized for stimulating T cells to initiate antigen-specific effector cell function [84–85]. During the functional maturation process, in response to inflammatory or microbial stimuli, changes occur in the profile of DC surface markers and cellular immune functions that define distinct immature versus mature immunofunctional phenotypes. Kv channels number among state-specific up-regulated transmembrane proteins known to play prominent roles in the cellular activation of a wide variety of immune system cells of both lymphoid and myeloid lineage.

In spite of the importance of DCs as immunoregulators of T cells, studies of DC Kv channels have only just begun. The presence of functioning Kv1.3 channels [47] was first described in murine DCs that were terminally matured and exhibited a high surface-membrane expression of MHC II molecules. Studies are currently under way examining human DCs throughout the full process maturation. Preliminary results indicated that a sequential and state-specific up- and down-regulation of three distinct Kv channels: first KIR, followed by Kv1.5, and ultimately Kv1.3 [57]. More detailed studies have since revealed that stimulated DCs express a mix of both Kv1.3 and Kv1.5 channels, with Kv1.5 predominating in matured DCs. Furthermore, these studies demonstrated that blockade of Kv1.3 and Kv1.5 impaired clusters of differentiation 83 (CD83), CD80, and CD86 up-regulation and interleukin 12 (IL12) and IL6 production, indicating that these channels play a functional role in DC maturation [48]. DCs are attractive alternate MS therapeutic targets to T cells for two reasons. First, DC stimulation and maturation precede DC-initiated stimulation of T cells. Second, DCs constitute a peripheral systemic (CSF, meninges, choroid plexus, and deep cervical lymph nodes), as well as a CNS (MS lesions) target for the development of future clinical treatments in MS. Thus, targeting select DC Kv channels to interfere with DC maturation may offer an early and unique opportunity to inhibit T cell effector function by aborting the induction of T cells as autoimmune effector cells in MS.

**TARGETING KV CHANNELS AS ANTI-INFLAMMATORY THERAPY**

The hallmark of neuroinflammation is a microglial or microglial/macrophage response that has been observed in several neurodegenerative diseases, including MS, making it reasonable to consider anti-inflammatory therapy for MS to inhibit microglial activation. Specifically, clinical benefits following anti-inflammatory treatment have been demonstrated in mice with a genetic motor-neuron disease in which microglia are prominent [86–88]. In another model of neuroinflammatory disease, PVC-211 murine leukemia virus (MuLV)-induced spongiform neurodegenerative disease in rats, a highly reactive microglial/macrophage response is associated with severe free-radical injury, motor neuron injury, and death. Vitamin E pretreatment of rat pups delays the appearance of free-radical injury and delays but does not inhibit disease expression [89]. Furthermore, minocycline, an antibiotic with inhibitory effects on macrophages and microglia, inhibits the reactive microglial/macrophage response and delays the expression of PVC-211 MuLV disease [90] and is effective in slowing the disease course in superoxide dismutase (SOD1)G93a mutant motor-neuron disease [88]. The presumed mechanism is inhibition of microglial/macroglial function. More recently, the cyclooxygenase-2 inhibitor celecoxib has been effective in slowing the disease course in SOD1G93a mice. This has led to an ongoing clinical trial of this compound in patients with Lou Gehrig’s disease. While these broadly reactive anti-inflammatory compounds may show partial effects in animal models and, we hope, in clinical trials, a need clearly exists for more targeted therapy. Thus, microglial and/or
macrophage Kv channels may represent a possible target for intervention.

Of the immune system cells considered integral to MS autoimmune processes, the study of Kv1.3 in activated microglia and macrophages has only recently garnered attention. Microglia play a central role in mediating CNS inflammatory processes and as the only resident brain immune system cells, activated microglia can proliferate, migrate to sites of injury, present antigen, phagocytize, secrete proinflammatory cytokines and cytotoxins, and undergo a nicotinamide adenosine dinucleotide phosphate (NADPH)-mediated respiratory burst producing cytotoxic reactive oxygen and nitrogen species.

Three lines of evidence suggest a central role for microglia in the disease processes leading to demyelination and irreversible axonal damage underlying conduction deficits in MS. First, active MS lesions contain reactive microglia [91–92]. Second, throughout active demyelinating lesions and along the borders of chronic active lesions [93], NOS catalytic activity is elevated, as are levels of NO, a proinflammatory reactive nitrogen-free radical generated by activated microglia [94–99]. Third, NO donors can produce reversible conduction block in normal and experimentally demyelinated axons and morphological changes consistent with acute Wallerian degeneration [100–101]. Thus, reactive microglia and a proinflammatory microglial activation product are implicated in the long-established conduction deficits and newly recognized axonal damage associated with MS.

As seen in other immune system cells (T cells and macrophages), Kv channels appear to regulate proliferation and cellular activation in microglia. Two distinct Kv channels are expressed differentially in microglia: Kv1.5 in resting, nonproliferating cells and Kv1.3 in activated, proliferating cells [58,102]. While Kv1.3 up-regulation has been associated with various effector cell functions following microglial activation [40,44,55], the precise role of Kv1.3 versus Kv1.5 channels in microglial function remains unclear. To date, Kv1.3 up-regulation is associated with granulocyte macrophage-colony stimulating factor, interferon-γ, and lipopolysaccharide-stimulated activation [40,44,55], transforming growth factor-β stimulated microglial deactivation [56], and the NADPH-mediated respiratory burst [43], a metabolic cascade, the products of which have been identified in MS [43,103–104].

**CONCLUSION: FUTURE POTENTIAL FOR TARGETING KV CHANNELS IN MULTIPLE SCLEROSIS**

Two mononuclear phagocytes, CNS microglia and peripheral DC, are critical players in CNS inflammation. As such, microglia and DCs are important immune cell targets for new MS therapies aimed at modulating cell function by blocking Kv channels. In the CNS, activated microglia are the primary effector cells underlying the immune-mediated pathogenesis of inflammation, demyelination, and breakdown of the blood-brain barrier (BBB) leading to neuronal injury and dysfunction [103]. Peripherally, mature DCs are essential for initiating and regulating primary T cell responses, which require peripheral stimulation to cross the BBB [105].

Given the known preferential Kv1.3 up-regulation in effector T cells, activated microglia and macrophages, and mature DCs, beneficial therapeutic effects resulting from the use of highly selective Kv1.3 blockers could arise from modulation of any or all of these immune cells. Even though highly selective peptide toxins have been identified that are better blockers of the Kv1.3 channel than 4-AP or 3,4-DAP, they are, at present, handicapped as viable therapeutics because of their short half-life of approximately 20 min [19]; synthetic toxin analogues are being developed to overcome such limitations [106].

Distinct from T cells and macrophages following stimulation, microglia up-regulate Kv1.5 during early stages of cellular activation prior to the up-regulation of Kv1.3 at terminal stages of activation, while DCs predominantly up-regulate Kv1.5 over Kv1.3 in their mature immunocompetent state. Although the precise functional roles of the Kv1.5 and Kv1.3 Kv channels remain unclear, their differential expression reveals Kv1.5 as an earlier and, thereby, potentially more important therapeutic target than Kv1.3 in microglia, and a primary target in DCs that distinguishes them from T cells. Studies to modulate the immune and neuroinflammatory response by affecting Kv1.5 and Kv1.3 activation are in progress in animal models. Translating these studies to MS offers a new therapeutic approach to this inflammatory neurodegenerative disease.

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