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Review

Ion channels in autoimmune neurodegeneration

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by widespread inflammation, focal demyelination and a variable degree of axonal and neuronal loss. Ionic conductances regulate T cell activation as well as neuronal function and thus have been found to play a crucial role in MS pathogenesis. Since present therapeutical approaches are only partially effective so far, ion channel modulation as a future strategy was brought into focus. Here, we review the status quo concerning recent findings from ion channel research in MS and its animal model, experimental autoimmune encephalomyelitis.
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1. Introduction

Multiple sclerosis (MS) is a neuroinflammatory disease associated with demyelination and neuronal cell death. As MS is the major cause of neurological disability in young adults, many costs and research efforts have been spent to identify potential therapeutic targets. However, MS therapy is only partially effective so far. Available drugs serve to suppress and modulate the immune response, but their impact on disease progression and permanent disability is only modest [1]. Accumulating neurological deficits are due to axonal and neuronal degeneration during the disease course. Several causes are under discussion: (1) during phases of acute inflammation, neuronal and axonal damage are caused by infiltrating immune cells that induce either direct cell–cell-interactions or transmitter release (NO, glutamate); (2) damaged oligodendrocytes lead to demyelination which in turn causes an insufficient trophic supply that finally results in axonal degeneration; (3) demyelination induces alterations in distribution and expression of different ion channels and transporters in axonal membranes. Accordingly, increased electrical activity as well as augmented intracellular Ca^{2+} levels result in a dysfunction of mitochondria and neuronal cell death in the end. Thus, it seems reasonable to ask: Does MS belong to the group of channelopathies? Originally, MS was conceived as a chronic inflammatory autoimmune disease of the central nervous system which is accompanied by demyelination that either delays or blocks electrical impulse conductance in central neurons. However, the characteristic accumulation of neurological deficits during MS disease course cannot solely be explained by de- and remyelination processes. Currently, it is considered to be due to axonal and neuronal degeneration. In this context, it can be assumed that one leading mechanism is the reorganization and altered gene expression of different ion channels which occurs in demyelinated axons. Therefore, MS may be defined as a member of the group of channelopathies, i.e., disorders in which abnormal ion channel function leads to the appearance of clinical signs and symptoms. These diseases can be further subdivided in genetic, autoimmune and transcriptional/translational channelopathies. (1) Genetic channelopathies are characterized by altered ion channel structures and function that are due to mutations in the channel genes [2]. (2) The autoimmune channelopathies are a group of neurological disorders in which the patient develops raised serum levels of highly specific autoantibodies to various neuronal or muscle ligand-gated or voltage-gated ion channels, or to related functional proteins [3]. (3) The third group is comprised of translational/transcriptional channelopathies which are disorders that are caused by a dysregulated production of normal channel proteins as a result of changes in the complex and highly dynamic process of gene transcription [4]. As a consequence, neuronal cell functions are perturbed. In terms of MS

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and its animal model experimental autoimmune encephalomyelitis (EAE), a number of different reports reveal their affiliation to the third subgroup of translational channelopathies in which it is shown that the pharmacological modulation of ion channel functions ameliorates disease course and clinical severity.

Studies on EAE revealed ion channels as being key players in pathophysiological processes of MS. Ion channels on neurons but also on immune cells strongly influence basic cellular parameters like the membrane potential and calcium signaling, and hence they regulate cell activity. According to their ubiquitous expression pattern, ion channels have the potential to influence nearly every stage of MS pathogenesis. The regulation of the immune response is fundamentally dependent on ion channels which are expressed on immune cells and which allow peripheral T lymphocytes to proliferate and to produce inflammatory cytokines [5]. Ion channels on neurons and glia cells affect the mechanisms that induce axonal and neuronal degeneration in white matter of the brain and spinal cord [6,7]. The application of different sodium, potassium and calcium channel inhibitors considerably ameliorates the EAE disease course as well as clinical severity, and it postpones the disease onset after immunization in comparison to sham-treated control animals [5,8,9]. Based on these results, ion channels seem to be promising in terms of therapeutic target structures. Nevertheless, the beneficial effect in these pre-clinical blocker studies may be mediated by two distinct pathways: either the activation/inhibition of an ion channel on nerve cells or T cells may facilitate neuronal survival or mediate an immune-modulatory effect. Thus, there is still a question to be resolved: Does a particular ion channel modulation strategy exert an immune-modulatory or a neuroprotective effect in MS?

2. Ion channels on T lymphocytes

A major contribution of human T lymphocytes to autoimmune inflammation in MS pathogenesis has already been suggested. However, the underlying pathophysiological processes are poorly understood. As basic cellular T cell functions like maintenance and modulation of the membrane potential depend on a number of different ion currents, ion channels were appraised as potential target structures for future pharmacological approaches. After antigen-specific T cell activation, a long-lasting increase in intracellular Ca^{2+} levels is needed to induce gene expression, proliferation and cytokine production. This influx of cations is outbalanced by hyperpolarizing potassium currents that preserve the driving force and a stable membrane potential. The great importance of ionic currents in regulating T cell effector functions is underlined by the fact that T cell activation leads to an upregulation of a number of different ion channel genes. After chronic stimulation, human cytotoxic $CD8^+$ T cells showed a close to 10-fold upregulation of mRNA coding for a member of the two-pore domain K^+ channel (K_{2P}) family [10].

2.1. K^+ channels

By now, a number of different ion channels on T cells are identified. There are three most prominent potassium channels: a voltage-gated K^+ channel [$K_V1.3$] [11,12], a Ca^{2+}-activated and intermediate conductance K^+ channel [$K_{Ca}3.1$ or IK_{Ca}] [13,14] as well as four distinct members of the two-pore K^+ channel family: TASK1, TASK2, TASK3 and TRESK [5,10,15,16]. In T cells under basal conditions, the resting membrane potential is maintained at about -50 mV by a K^+ outward current carried by $K_v1.3$ channels. There are numerous studies showing that inhibition of $K_v1.3$ offers a promising approach to modulate pathological immune responses mediated by autoreactive effector memory T cells (T_{EM}). Selective $K_V1.3$ inhibitors do not prevent immunological synapse formation, but they suppress Ca^{2+} signaling, cytokine production and proliferation of autoantigen-specific T_{EM} at pharmacologically relevant concentrations while sparing other classes of T cells [17,18]. Although there was an apparent lack of immune system defects due to counterbalancing chloride currents in $K_V1.3$ knockout mice [19], selective blockade of $K_V1.3$ channels in miniature swines reduced the immune response in vivo [20]. $K_v1.3$ expression was found to be high in T cell clones of the appropriate antigen specificity of MS patients [21]. In addition, $K_v1.3$ positive cells were highly evident in the immune infiltrates of the majority of MS plaques [22].

A second type of K^+ current in T cells is carried by Ca^{2+}-activated and intermediate conductance K^+ channels ($K_{Ca}3.1$). Based on their K^+ conductance in the range of 20–40 pS, they can be distinguished from the closely related small ($K_{Ca}2.x$; 5–20 pS) and large conductance ($K_{Ca}1.1$; 200–250 pS) K^+ channels [23]. $K_{Ca}3.1$ channels are closed under resting conditions with low basal cytosolic Ca^{2+} levels and open rapidly if Ca^{2+} rises intracellularly. Thus, these channels support the maintenance of the membrane potential that allows the long-lasting Ca^{2+} influx after T cell receptor stimulation. Blocker studies with a highly specific blocker of these K^+ channels (TRAM-34) showed that $K_{Ca}3.1$ channels play a critical role in the immune response during the development of MOG-induced EAE in C57BL/6 mice. However, the effect of TRAM-34 application was reversible, as indicated by the development of clinical EAE symptoms within 48 h after withdrawal of treatment [9]. Additionally, the efficacy of K^+ channel blockers was assessed in rats by transferring donor lymphocytes from rats with EAE to healthy, untreated recipients [adoptive transfer (AT)-EAE] [25]. Specific and simultaneous inhibition of $K_v1.3$ and $K_{Ca}3.1$ channels by a $K_v1.3$ blocker (ShK) or by a combination of a highly specific Shk analogue (ShK-Dap22) plus TRAM-34 prevented lethal AT-EAE. Blockade of $K_v1.3$ alone with ShK-Dap22, but not of $K_{Ca}3.1$ with TRAM-34, was also effective. When administered after the onset of symptoms, ShK or the combination of ShK-Dap22 plus TRAM-34 greatly ameliorated the clinical course of both moderate and severe AT-EAE [24].

In human and murine T lymphocytes, three distinct members of the two-pore potassium channel family are constitutively expressed: TASK1, TASK2 and TASK3. In addition, the expression of the calcineurin-regulated K_{2P} channel TRESK was postulated in leukemic Jurkat T cells [16]. The functional role of TRESK in primary T cells still needs to be confirmed. However, pharmacological inhibition of TASK1, TASK2 or TASK3 leads to a reduction in cytokine production and proliferation after T cell receptor stimulation. In vivo relevance of T cells can be tested by AT-EAE. Pharmacological blockade of TASK channels on myelin basic protein (MBP)-specific T cells before transfer resulted in a significant amelioration of the disease course [15]. After induction of myelin oligodendrocyte glycoprotein (MOG)-EAE, TASK1 knockout mice showed a significantly reduced clinical severity and markedly reduced axonal degeneration compared to control animals. Stimulated T cells from TASK1 knockout animals showed impaired cell proliferation and cytokine production, while the immune repertoire and naïve T cells were otherwise normal. Supportingly, pharmacological inhibition by the endocannabinoid anandamide (a semi-selective inhibitor of TASK channels) reduced $IFN\gamma$ secretion in stimulated T cells from wild-type but not from TASK1 knockout animals. Additionally, application of anandamide protected EAE animals from severe brain damage which was assessed by MRI [5]. Electrophysiological data revealed a significant contribution of TASK channels to the total outward current of T cells [15].

2.2. Calcium-release activated calcium channels, CRAC

The prolonged Ca^{2+} influx, which is obligatory for the induction of gene expression after antigen-specific stimulation, is mainly generated by Ca^{2+}-release activated Ca^{2+}(CRAC) channels [26,27]. In lymphocytes, CRAC channels are physiologically triggered by

Table 1

Overview of ion channels expressed on T cells. The table comprises channels which are shown to be expressed in primary T cells. Signs: (+ → +), unaltered gene expression; (+ → ++), upregulated gene expression; (+ → +++), strongly upregulated gene expression; (+ → − − −), downregulated gene expression. Abbreviations: ASSC, amiloride-sensitive sodium channel; CRAC, Ca^{2+}-release activated Ca^{2+} current; IFNγ, interferon-gamma; IL, interleukin; K_{Ca}, calcium-activated potassium channel; K_V, voltage-gated potassium channel; LT, lymphotoxin; MIC, Mg^{2+}-inhibited Ca^{2+}-permeable current; stim, stimulated; unstim; unstimulated; TASK, TWIK-related acid-sensitive K^+ channel; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; TNFα, tumor necrosis factor-alpha; T_{reg}, regulatory T cell; TRPM7, a member of the transient receptor potential melastatin-like (TRPM) ion channel subfamily; VRAC, volume-regulated anion current.

Ion selectivity	Channel name	Human gene name	Expression (unstim → stim)	Remarks, effects	References
K^+	TASK1, $K_2P3.1$	KCNK3	T cells	• Proliferation • IFNγ, IL-2, IL-4, IL-5 and IL-10 production • T cell invasiveness • Axonal degeneration	[5,15]
	TASK2, $K_2P5.1$	KCNK5	$CD4^+$cells (+ → +++) $CD8^+$cells (+ → +++)	• Proliferation • IFNγ production	[10]
	TASK3, $K_2P9.1$	KCNK9	T cells	• Proliferation • IFNγ and IL-2 production	[15]
	$K_V1.1$	KCNA1	$CD4^-CD8^-$ cells $CD4^+$ cells (+ → − − −) T_{EM}	• In mouse T cells • Thymocyte development	[33,34]
	$K_V1.2$	KCNA2	$CD4^+$cells (+ → − − −)	• In mouse T cells	[33]
	$K_V1.3$	KCNA3	Naïve $CD4^+$ cells (+ → +) Naïve $CD8^+$ cells (+ → +) T_{reg} $CD4^+/CD8^+$ T_{CM} (+ → +) $CD4^+/CD8^+$ T_{EM} (+ → +++)	• Proliferation • Immunological Synapse • IL-2, TNF production	[11,21,22,35–39]
	$K_V1.6$	KCNA6	$CD4^+$ cells (+ → − − −)	• In mouse T cells	[33]
	$K_{Ca}3.1$	KCNN4	Naïve $CD4^+$ (+ → +++) Naïve $CD8^+$(+ → +++) $CD4^+/CD8^+$ T_{CM} (+ → +++) $CD4^+/CD8^+$ T_{EM} (+ → +) T_{reg}	• IFNγ, TNFα, IL-1, LT production • Proliferation	[9,14,21,37,40–43]
Cations, non-selective	TRPM7	TRPM7	T cells	• Mg^{2+} homeostasis	[28]
Ca^{2+}	CRAC	ORAI1	T cells (+ → +++)	• Store-operated Ca^{2+} entry • Gene expression • IFNγ, IL-2 production • Immunological synapse	[26–29,44]
	IP_3R	ITPR1-3	T cells	• Store-operated Ca^{2+} entry • Gene expression	[45,46]
Cl^-	VRAC	Unknown	T cells (+ → +)	• Regulated volume decrease	[47,48]
Na^+	ASSC	SCNN1	T cells (ENaCγ)	• Unknown	[49,50]

inositol-triphosphate (IP_3)-induced depletion of the endoplasmic reticulum (ER) Ca^{2+} store, followed by STIM proteins that physically convey the signal from the ER to the plasma membrane, induce the opening of CRAC channels and allow Ca^{2+} ions to enter the cell [28]. Solely CRAC channels provide the prolonged Ca^{2+} influx into lymphocytes, but unfortunately in absence of a specific CRAC channel blocker only little experimental data about the in vivo function of these channels has been reported so far. However, a knock-in mutation of one CRAC encoding gene Orai1 causes the expression of a non-functional ORAI1-R93W protein. The affected mice show normal lymphocyte development, but T and B cells display severely impaired store-operated Ca^{2+} entry and CRAC channel function resulting in strongly reduced expression of several key cytokines [29]. Moreover, genetic deletion of STIM1/2 ameliorates the disease course of EAE [30,31] and a human immunodeficiency syndrome associated with deficiencies in STIM1 function was described recently [32].

In summary, T cell activity involves a complex interplay of different ionic currents that regulate basic T cell effector functions like cytokine secretion and cell proliferation induced by T cell receptor stimulation. The broad variety of different potassium channels that are expressed on T cells point to a scenario in which the function of potassium channel activity goes beyond the maintenance of the membrane potential. However, a potential contribution to intracellular signaling cascades is unknown so far (Table 1).

3. Ion channels on nerve cells

Classically, the central nervous system was considered an immune-privileged organ. However, in case of neuroinflammatory diseases like MS and EAE, nerve and immune cells get into contact. Besides the influence of ionic conductances of immune cells, numerous studies revealed that ion channels expressed on neurons also contribute to the pathophysiological scenario during neuroinflammation.

3.1. Voltage-gated sodium channels

Under healthy conditions, the voltage-gated sodium channel $Na_v1.2$ is expressed on immature, unmyelinated neurons. As such, they support the continuous impulse transmission, but they are no longer detectable on adult, myelinated neurons. The closely related $Na_v1.6$ channels support the saltatory conductance of the nerve impulse at nodes of Ranvier of adult neurons. However, in case of demyelination expression and distribution of voltage-gated sodium channels at neuronal somata and axons are completely altered. $Na_v1.2$ and $Na_v1.6$ are found to be overexpressed at demyelinated sites of the axonal membrane [51]. While $Na_v1.2$ supports the restitution of the continuous form of signal conductance, $Na_v1.6$ mediates a persistent sodium inward current that finally induces the reversal of the Na^+/Ca^{2+} exchanger of the demyelinated membrane. The intracellular Ca^{2+} accumulation triggers mitochondrial dysfunction and leads to neuronal cell death in the end [51]. Based on these facts, the blockade of voltage-gated sodium channels (especially $Na_v1.6$) seems to be promising in terms of a therapeutic target that might allow neuronal survival and thus reduce permanent neurological deficits of MS patients. Inhibition of sodium channel function using specific blockers revealed that two distinct blockers, phenytoin and carbamazepine, significantly improved the clinical course of the disease. However,

withdrawal of treatment resulted in acute exacerbation, accompanied by a significantly increased inflammatory infiltrate within the central nervous system and the death of nearly 60% of phenytoin-treated and 8% of carbamazepine-treated EAE mice [52]. In a phase 2 trial, the neuroprotective role of lamotrigine, a partial sodium channel blocker, was tested in patients with secondary progressive multiple sclerosis (SPMS). The effect of lamotrigine on cerebral volume of SPMS patients was investigated by means of MRI, and it did not differ from that of placebo over 24 months. However, lamotrigine seemed to cause early volume loss that reversed partially on discontinuation of treatment [53]. Another clinical trial with phenytoin in patients with primary progressive MS was planned, but due to the clinical worsening after withdrawal in the animal study mentioned above [52], the study was never initiated [54,55]. Although the findings in pre-clinical studies pointed to an effective mechanism to ameliorate MS symptoms, sodium channel blockers by now missed the clinical translation due to the rebound effect after discontinuation of the drug.

3.2. Voltage-gated calcium channels

Several lines of evidence support the notion that aberrations in ionic currents carried by voltage-gated calcium channels contribute to the pathophysiology in MS and EAE. Increased influx of extracellular calcium through voltage-gated calcium channels (VGCC) was assumed to facilitate the neurological impairment and pathological outcome in EAE, and by inference, MS. The expression of the pore forming α_{1B}-subunit of N-type calcium channels ($Ca_v2.2$) was investigated in MS and EAE plaques and was found to be overexpressed in active lesions [56]. By means of electron microscopy, these α_{1B}-subunits were found to be located in the axonal membrane and thus might mediate an increased neuronal calcium influx. Based on these findings and together with the fact that calcium channels are present on oligodendrocytes [57] and axons [58] – both key targets in autoimmune demyelination –, EAE mice were treated with two different calcium channel blockers, bepridil and nitrendipine. Both drugs, although different in many structural and functional respects, block calcium influx through L-type calcium channels ($Ca_v1.2$, $Ca_v1.3$, $Ca_v1.4$). As compared to placebo-treated controls, these calcium antagonists had comparable beneficial effects with reduced inflammation and axonal pathology on AT-EAE mice [59]. As largely increased intracellular calcium levels are known to directly trigger apoptotic signals and neuronal degeneration, calcium channels also seem to play a role in pathological mechanisms of inflammatory demyelinating diseases. Nevertheless, there are no trials for clinical translation of calcium channel blockers so far.

3.3. Voltage-gated and K_{2P} potassium channels

Intact electrical impulse transmission includes different potassium conductances that are carried by voltage-gated and calcium-activated potassium channels. At an intact, myelinated axon, the voltage-gated potassium channels $K_v1.1$ and $K_v1.2$ are located underneath the myelin sheath in the paranodes or internodal regions. They affect the electrotonic conductance and repolarisation of the action potential generated at the node by mediating K^+ outward currents [60]. Glia cells also possess inwardly rectifying potassium channels ($K_{ir}4.1$) that support the potassium homeostasis in the extracellular space [7]. Demyelination induces alterations in K^+ channel expression and distribution, as there is an upregulation of $K_v1.1$ and $K_v1.2$ on axons and of $K_v1.4$ on glia cells. Due to increased K^+ outward flux from axons and a decreased K^+ buffering by glia cells, the extracellular K^+ homeostasis is outbalanced and postpones the K^+ reversal potential to more positive values. As a consequence, there is a gradual depolarisation of the membrane

potential which induces an inactivation of voltage-gated sodium channels and hence signal conduction block. The axonal K^+ outward currents lead to an intracellular potassium depletion that is assumed to be a first step in apoptotic cascades as it triggers water loss and disinhibition of intracellular proapoptotic enzymes [61]. Administration of a $K_v1.1$ selective blocker (BgK-F6A) ameliorates disease course in EAE mice while it did not affect T cell activation [62]. In vivo relevance of TASK channels on T lymphocytes was addressed by pre-treatment of myelin basic protein-specific encephalitogenic T lymphocytes with a TASK1 modulator (anandamide) which was associated with significant amelioration of the disease course in Lewis rats [15]. Adding MOG-reactive T lymphocytes onto acute living brain slices resulted in a significantly higher number of apoptotic neurons in wild-type slices compared with TASK1$^{-/-}$ preparations [5]. Based on these recent findings about the contribution of TASK channels to EAE pathology, there is good evidence that inhibition of TASK channels exerts an immunsuppressive as well as a neuroprotective effect. Thus, these two pore domain K^+ channels could function as a new therapeutic concept in therapy of neuroinflammatory disorders, but clinical trials are currently hampered by the lack of available highly specific blockers.

Taken together, differently from clearly defined channelopathies like cystic fibrosis, the MS pathology seems to be influenced by multiple ion channels which include different ion selectivities and which can be expressed both on neurons and on T lymphocytes. This indicates a highly complex scenario of MS pathogenesis which should be taken into consideration while planning trials for clinical translation of newly discovered therapeutic agents. Apart from the postulated mechanism of action, there might be numerous "off-target" effects in human beings, although convincing pre-clinical studies in murine animal models and non-human primates have been performed in advance [1].

4. Differentiation between immune-suppressive/immune-modulatory and neuroprotective drug actions

In MS, myelin and neurons are lost as a result of an inflammatory attack on the central nervous system (CNS). The lesions alter nerve conduction and contribute to disabling neurological deficits that vary with the location of demyelination plaques within the CNS. Although the cause of MS remains undetermined, the presence of large immune infiltrates in the white matter of patients suggests that myelin damage is immune-mediated. T and B lymphocytes are major components of these infiltrates whereas it is accepted that autoimmune T cells mediate the early steps of new multiple sclerosis lesions [63,64]. A number of experimental studies showed that the application of ion channel blockers to EAE animals induces attenuated disease courses and impaired clinical severity as well as delayed onset of symptoms. However, in most cases the exact mechanism of blocker action could not be elucidated, since many of the blocked ion channels are expressed on neuronal and immune cells as well as on the myelin-producing oligodendrocytes. A differentiation between the consequences of ion channel modulation on neurons and on immune cells would be beneficial to get further insights into pathophysiological processes and mechanisms of drug actions (Fig. 1).

4.1. ASIC1 – a neuroprotective Na^+ channel

A sophisticated strategy to differentiate between an immune-suppressive and neuroprotective drug effect can be a combination of adoptive transfer-EAE with specific ion channel deficient animal models. Accordingly, Friese and colleagues used mice with a genetically inactivated gene that encodes the neuronally expressed, proton-gated acid-sensing ion channel-1 (ASIC1) and compared them with wild-type mice (C57BL/6). After immunization with MOG
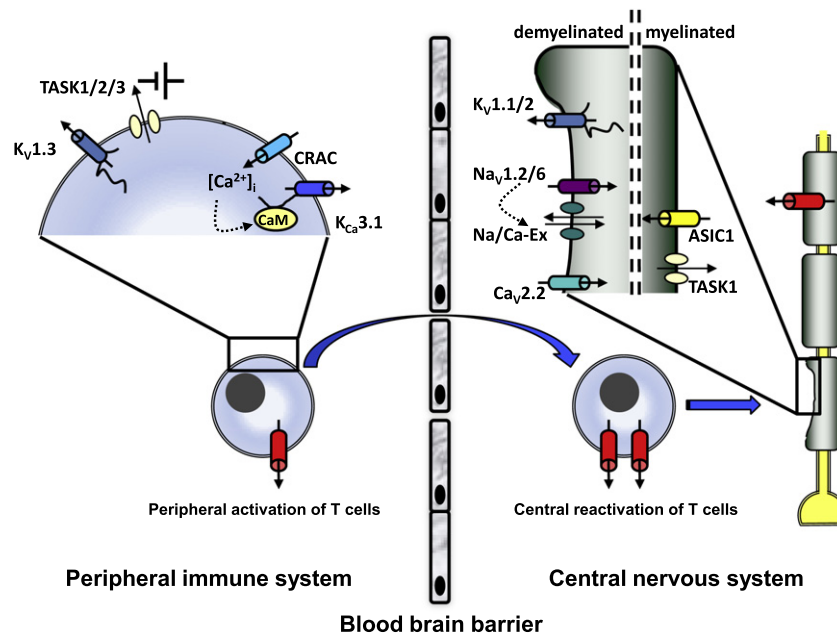
Fig. 1. Ion channels on T cells and in the CNS which are involved in the pathophysiology of MS are depicted on the left side (T cells) and right side (myelinated and demyelinated neurons). See text for an extensive description of the putative role of these channels. Abbreviations: ASIC, acid sensing ion channel; $[Ca^{2+}]_i$, intracellular calcium concentration; CaM, Calmodulin; Ca_V, voltage-gated calcium channel; CRAC, Ca^{2+}-release activated Ca^{2+} current; K_{Ca}, calcium-activated potassium channel; K_V, voltage-gated potassium channel; Na_V, voltage-gated sodium channel; Na/Ca-Ex, sodium–calcium exchanger; TASK, TWIK-related acid-sensitive K^+ channel.

peptide, ASIC1 knockout animals had a significantly lower clinical severity at disease maximum and an ensuing clinical deficit. To confirm that the disease-modifying effect of inactivated ASIC1 is T cell independent, effector T cells specific for MOG peptide and isolated from wild-type and ASIC1 knockout mice were transferred into naïve wild-type or ASIC1 knockout animals. Solely the disease course of ASIC1 knockout mice, that received MOG-specific T cells from wild-type or ASIC1 knockout donors, was ameliorated to an equal degree in both cases. Thus, these authors were able to clearly identify the blocked/ablated Na^+ channel ASIC1 to facilitate neuroprotection in AT-EAE animals, and they showed that this effect was most likely neuron-dependent [6,65].

4.2. TASK1 modulates immune response and neurodegeneration

By applying an alternative approach, recent results from our lab identified neuronally expressed TASK1 channels to exert a protective impact on neurons in EAE animals [5]. In TASK1 deficient mice ($TASK1^{-/-}$), experimental autoimmune CNS inflammation was significantly attenuated and was accompanied by a markedly reduced production of cytokines (IL2, IFNγ) of type 1 T helper cells (T_h1). Absence of TASK1 did not affect immune subset distribution and the phenotype of antigen-presenting cells, but it drastically impaired antigen-induced T cell proliferation and cytokine secretion. Moreover, immunological detection of SMI32 positive axons in cervical spinal cord sections revealed a beneficial influence of TASK1 deletion on axonal degeneration under inflammatory conditions in EAE mice. To directly show the positive effect being due to TASK1 channels expressed on neurons, acute living brain slices were incubated for 6 h with MOG-reactive CD4$^+$ T lymphocytes. A higher number of apoptotic neurons in wild-type slices compared to $TASK1^{-/-}$ preparations indicated a direct impact of TASK1 channels on neuronal survival and that the absence of TASK1 protected against immune-mediated axonal degeneration in EAE. These results and other lines of evidence point to TASK1 channels as being critical modulators of T cell immunity and neurodegeneration. Hypoxia and tissue acidification, that are characteristic conditions for an inflammatory environment as it occurs in EAE brain

and in spinal cord tissue [6], modulate TASK1 channel function [66–69]. Based on these results, on the unique pharmacological profile of TASK1 and on the knowledge about its extensive actions on basic cellular processes, it is assumed that TASK1 channels on neurons could push apoptotic mechanisms. An increased loss of potassium ions is known to be a first step in apoptosis which is followed by a loss of intracellular water or can be directly coupled to the activation of proapoptotic enzymes [70]. In agreement with these findings, levels of a semi-selective TASK channel blocker, the endocannabinoid anandamide, was shown to be elevated in MS plaques [71]. This fact points to an endogenous strategy to support neuronal survival during MS attacks.

5. Conclusion

Currently approved drugs for multiple sclerosis have mostly broad effects on the immune system (e.g., interferons, glatiramer acetate, mitoxantrone). Newly emerging drugs follow the same principle apart from their selective and defined target structures such as alpha4-integrin for natalizumab or sphingosin 1-phosphate receptor for fingolimod [72–75]. Ion channels have long been recognized as attractive target structures for other diseases as ligand-gated and voltage-gated ion channels can both be found among the top five pharmaceutical target groups of FDA-approved agents [76]. A diverse selection of ion channels from different protein families (e.g., voltage-gated Na^+-/K^+-/Ca^{2+} channels, epithelial Na^+-/K^+ channels and TRP channels [77]) provides further evidence that ion channels per se should be regarded as putative promising new targets for MS. This is especially the case for MS subtypes with a strong degenerative component (primary progressive and secondary progressive MS) with a strong need for novel therapies. However, the clinical translation of ion channel modulation remains a major challenge for MS. So far, only few larger clinical trials (e.g., lamotrigine) have been performed and most approaches are still in an early preclinical stage. Common reasons for this delay are a lack of highly specific ion channel inhibitors on one side (as for example for the family of K_{2P} channels) and a lack of knowledge about the exact role of ion channels in the context of

autoimmunity as can be seen in case of the phenytoin/carbamaze-pin withdrawal side effects.

A major advantage of pharmacological targeting of ion channels in autoimmune degeneration is their ubiquitous expression on all cells with very heterogeneous functions. Therefore, a specific selection of the appropriate ion channel could influence various aspects of multiple sclerosis. Blockade of $K_V1.3$ has been postulated as a potential immunosuppressive strategy for MS [21,22,78]. However, clinical results are still pending and a number of questions remains open (e.g., expression of $K_V1.3$ on other cell types, crossreaction of channel blockers with closely related $K_V1.x$ subtypes or long-term effect of $K_V1.3$ blockade). Blockade of TASK channels on T cells might prove beneficial as well, however, the pharmacological development of specific K_{2P} blockers is still unsatisfactory. As the family of K_{2P} channels has been discovered only 15 years ago, further development can be expected in this direction.

Another important target of ion channel modulation is the prevention of neuronal cell death and neurodegeneration. A clinical trial using amiloride as an unspecific ASIC1 blocker was announced in Oxford and a number of other promising targets (TASK1, $Na_V1.2$/6) have not been addressed in clinical trials yet. As recent research for MS has repetitively emphasized the importance of early and ongoing neurodegeneration – partly independent from inflammation – in MS, the therapeutic impact of addressing ion channels which directly influence neurodegeneration might be very attractive. It can be expected that a number of clinical trials will be started in the next years which will hopefully not only expand our knowledge about ion channel physiology but also lead to new treatment strategies for autoimmune diseases.

The identification of ion channels as new target structures in MS may also facilitate the search for genetic alterations or polymorphisms of the underlying genes that lead to disease generation. This may help to establish genetic testing for MS susceptibility and choosing effective drugs for treatment.

Acknowledgements

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