PERSPECTIVE

Orexin/hypocretinin in multiple sclerosis and experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is a T-cell-mediated autoimmune disease of the central nervous system (CNS). Worldwide, more than 2.3 million people are diagnosed with MS. Since its clinical manifestations appear typically in the third and fourth decades of life, MS is a major cause of neurological disability in young adults and has wide health, psychological, economic, and social consequences. There are three key pathological features of MS: inflammation; demyelination and oligodendrocyte loss; axonal loss and neurodegeneration. There are two main hypotheses regarding mechanisms of MS pathology. The "outside-in" concept is an older, widely recognized hypothesis that describes neurodegeneration as a consequence of inflammatory induced demyelination, which is caused by immune system activation (Lassmann et al., 2012). Mechanisms of T-cell mediated myelin destruction are extensively studied, but the manner by which the immune system perceives myelin as foreign, and induces an autoimmune response is still unknown. The newer, "inside-out" hypothesis considers MS to be a primary degenerative disorder, which initiates in oligodendrocytes and results in neuroinflammation that leads to demyelination (Stys et al., 2012). As one of the main pathological features of MS-pathology, induced neurodegenerative processes are present in different brain regions, including the hypothalamus (Hyp).

Many Hyp-regulated physiological functions are affected in MS, yet dysfunction of the Hyp is not typically thought to be part of the underlying etiology of MS, nor is it considered in treatment of MS symptoms. Common MS symptoms, such as fatigue, sleep impairment and weight dysregulation are associated with malfunction in one of the major neurotransmitter systems in the Hyp, the orexins (Burfeind et al., 2016). Orexins are hypothalamic neurotransmitters produced by a population of neurons predominantly located in the lateral hypothalamus. Orexin is present in two isoforms (orexin A and orexin B) and it binds to two variants of orexin receptors (G protein coupled receptors, orexin receptor 1 (OxR1), and orexin receptor 2 (OxR2)) (Wang et al., 2018). Orexin isoforms are present in most vertebrates (orexin is highly conserved between humans and mice) and apparently absent in invertebrates. Orexin receptor 1 binds orexin A with high affinity and it has a significantly lower affinity for orexin B, while OxR2 is less selective, binding both orexin isoforms with relatively high affinity. Binding of orexin to orexin receptors lead to activation of at least three different subtypes of G proteins (Gq, Gi, Gs) or other proteins such as β-arrestin and adenylyl cyclase resulting in regulation of phospholipases, ion channels and protein kinase activity. Intracellular calcium (Ca^{2+}), as a second massager, plays an important role in OxR-mediated pathways. Increases in Ca^{2+} concentration are regulated by the phospholipase C signaling cascade as well as via transient receptor potential channel 3, a type of nonselective cation channel. Orexin receptors activate signaling pathways include phospholipase D, phospholipase A, protein kinase B, 5′adenosine monophosphate-activated protein kinase, and extracellular signal regulated kinases cascades (Wang et al., 2018) (**Figure 1**). The most characteristic feature of orexin neurons is that they project to many different brain regions including cortical areas, hippocampus, striatum, and spinal cord. Even though orexin neurons are moderate in numbers (the human brain contains 60,000–80,000 orexin-producing neurons), they are involved in regulation of many seemingly unrelated processes such as motor function, cognition, anxiety, sleep, alertness, pain, etc. Orexin neurons receive strong inputs from brain regions responsible for stress, autonomic tone responses, reward and motivation, and circadian rhythm, and orexins regulate neuroendocrine functions.

In support of the idea that orexins may be involved in MS, the orexin system is impaired in MS (Burfeind et al., 2016); orexin has anti-inflammatory and neuroprotective properties; and orexins ameliorate experimental autoimmune encephalomyelitis (EAE) pathology (Becquet et al., 2019). Since MS is driven by an auto-immune attack against the myelin insulation of neurons it is believed that inflammation plays

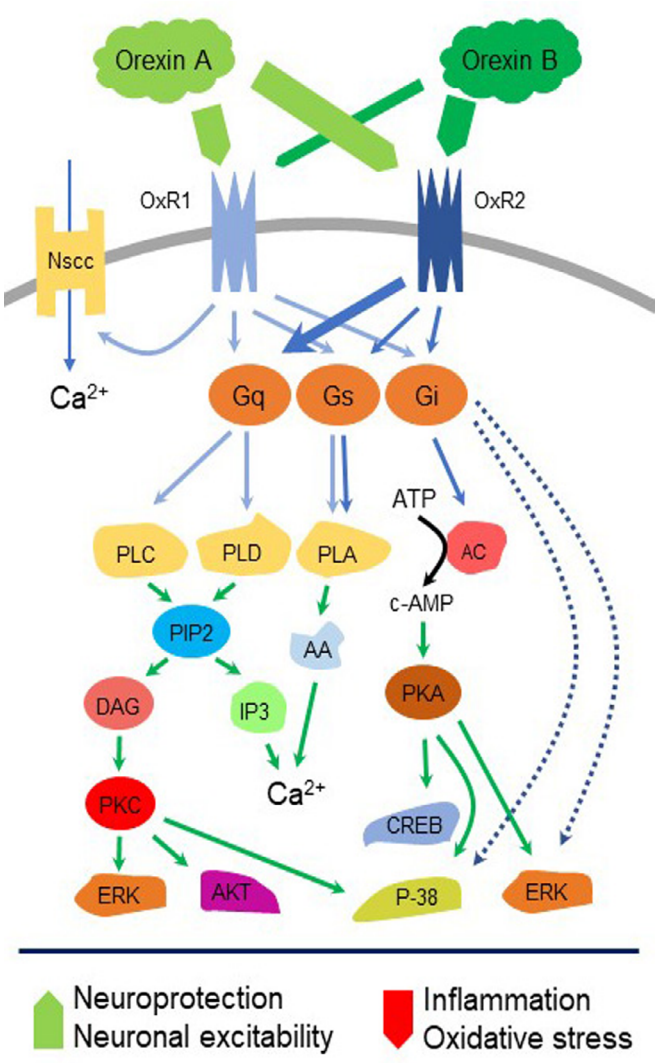

Figure 1 Orexin/receptor system mediated signaling.
The binding of orexin A and orexin B to orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2) leads to activation of Gq, Gi, and Gs; G protein subtypes. Activation of G proteins leads to activation of phospholipase C (PLC), phospholipase D (PLD), phospholipase A (PLA), adenylyl cyclase (AC), or protein kinase A (PKA). The activation of the named kinases and downstream molecules including phosphatidylinositol 4,5-bisphosphate (PIP2), inositol trisphosphate (IP3), arachidonic acid (AA), diacylglycerol (DAG), and protein kinase C (PKC) results in extracellular signal regulated kinases (ERK), mitogen activated protein kinases (P-38), protein kinase B (AKT) activation, and increase in cytosolic Ca^{2+}. OxR1 activates nonselective cation channels (NSCCs), leading to extracellular influx of Ca^{2+}.

a major role in MS pathology. Immune system microglial cells and macrophages are important sensors of CNS pathology. In MS, myelin reactive T cells produce large quantities of T helper cell 1 cytokines that activate microglial cells and macrophages which in turn produce pro-inflammatory cytokines and chemokines, reactive oxygen, and reactive nitrogen species. This leads to amplification of inflammation and causes damage that leads to demyelination and neurodegenerative processes. Orexin A treatment reduces production of reactive oxygen species as well as interleukin (IL)-1β, IL-6, and IL-8 expression. Orexin A inhibits tumor necrosis factor-α induced activation of the nuclear factor-κB signaling pathway. Inhibition of the nuclear factor-κB signaling pathway leads to reduction of the expression of matrix metalloproteinase-3 and matrix metalloproteinase-13 enzymes, which are responsible for the breakdown of extracellular matrix proteins during tissue remodeling and transmigration of inflammatory cells from the

vasculature to tissue inflammation site (Sun et al., 2018). Finally, orexin promotes glucocorticoid secretion and activates the sympathetic nervous system by increasing release of catecholamines, which have anti-inflammatory properties and suppress the immune system (Grafe and Bhatnagar, 2018).

Several studies report that orexin A has neuroprotective properties as well. Orexin A reduces neurodegeneration in models of cerebral ischemia, and neurodegeneration induced by severe oxidative stress. Orexin A is neuroprotective in Parkinson's disease induced neurodegeneration (Becquet et al., 2019) and orexin receptor activation is neuroprotective in Alzheimer's disease via heterodimerization with G-protein coupled receptor 103 (Davies et al., 2015).

Several pre-clinical studies on EAE (the most commonly used model for MS) support the idea of orexin as a potent anti-inflammatory agent. A few years ago, Fatemi et al. (2016) investigated the effects of orexin A on clinical symptoms of EAE. Locomotor activity, exploratory behavior, pain sensitivity, as well as histology and expression of several genes involved in EAE pathology were observed. The authors reported that intraventricular injections of orexin A significantly attenuated the clinical symptoms of EAE and increased latency response in the hot-plate test. Further, orexin A intraventricular injections inhibited infiltration of inflammatory cells and up-regulated gene expression of transforming growth factor β, which in context can be considered as an anti- or pro-inflammatory marker. The expression of myelin basic protein (a major component of myelin sheets) was increased as well, suggesting a reduction in demyelination and/or enhanced remyelination. Orexin A reduced gene expression of the nitric oxide synthase, an oxidative stress marker which actively controls EAE pathology in the CNS. Finally, matrix metalloproteinase 9 (modulator of inflammation) and IL-12 (pro-inflammatory cytokine) expression were downregulated, supporting the hypothesis of orexin A anti-inflammatory effects in EAE. Another recent study addressed the effects of systemic orexin A and neuroinflammatory processes in EAE (Becquet et al., 2019). In this study, orexin A treatment reduced the EAE clinical score as well as demyelination, microglial activation, and astrogliosis. Furthermore, orexin treatment limited the infiltration of pathogenic $CD4^+$ T lymphocytes and did not impair peripheral draining lymph node cell proliferation and T helper cells 1 and 17 cytokine production in response to myelin oligodendrocyte glycoprotein (MOG35–55) *in vitro*. Finally, orexin A treatment diminished expression of monocyte chemoattractant protein 1, interferon gamma-induced protein 10 pro-inflammatory chemokines, interferon γ, IL-17, tumor necrosis factor-α, IL-10, and transforming growth factor β pro-inflammatory cytokine in the CNS.

There is scarce but interesting evidence of orexins and orexin system involvement in MS. Some of the common symptoms associated with MS are fatigue and sleep disturbances (Bøe Lunde et al., 2012). Sleep impairment is common in MS patients and studies show that ~50–65% of patients experience disturbed sleep. Interestingly, the physiological and psychological effects of MS are more severe in MS patients with sleep impairment (Bøe Lunde et al., 2012). Subjective sleep disorders in MS patients are closely associated with fatigue symptoms. Furthermore, studies propose that fatigue affects up to 83% of MS patients, making fatigue one of the most common symptoms in MS (Manjaly et al., 2019). As the orexin system is a major regulator of sleep and fatigue, it seems likely there is a potential involvement of the orexin system in MS. Indeed, there are several case reports of MS patients with Hyp lesions and low cerebrospinal fluid orexin levels, and accompanying hypersomnia or fatigue, as well as cohort studies of MS patients showing altered levels of orexin associated with fatigue (Burfeind et al., 2016; Manjaly et al., 2019). Further, reductions in orexin A accompanied by narcolepsy has been observed in MS patients as well as individual cases of patient with MS-pathology associated Hyp lesions, proposing the presence of gradual orexin neuronal loss during the disease course (Burfeind et al., 2016; Gencer et al., 2019). Moreover, Gencer et al. (2019) suggested that decreased orexin-A levels accompanies MS progression and motor system deterioration in the earlier stages of the disease and proposed that orexin-A might be used as a potential biomarker of physical disability in MS. However, it must be stressed that causes of poor sleep and fatigue in MS patients are most likely multifactorial and include adverse effects in combination with therapies and MS-associated symptoms, which may be caused, at least partially, by orexin system impairment.

Although the "outside-in" and "inside-out" hypotheses are conflicting, they both propose that MS therapy should be based on anti-inflammatory, neuroprotective, and regenerative strategies. The Hyp is centrally involved in various homeostatic processes, many of which are disrupted in MS patients. Orexin, having both immuno-modulating and neuroprotective properties, supported with the fact that the orexinergic system might be involved in the pathological development of MS, identifies this neurotransmitter as an interesting target for symptomatic sleep disorder, fatigue and cognitive impairment, as well as anti-inflammatory therapy for MS.

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