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Enhancing Remyelination through a Novel Opioid-Receptor Pathway

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Review of Mei et al.

The evolution of myelin 488 million years ago was a critical step for vertebrate diversification, co-occurring with the development of jaws and primary components of the brain (Stiefel et al., 2013). Myelin is vital for biological fitness because it increases the efficiency and conduction velocity of action potentials while simultaneously providing metabolic support for axons (Saab et al., 2016). The production of new CNS myelin is indispensable for learning new motor skills in both juveniles and adults (McKenzie et al., 2014; Xiao et al., 2016). Any injury, disease, or disorder that results in chronic demyelination can lead to debilitating conditions, such as multiple sclerosis (MS), leukodystrophies, Guillain-Barré syndrome, and Charcot-Marie-Tooth disease.

CNS myelin is produced exclusively by postmitotic oligodendrocytes, which develop from an endogenous pool of cycling oligodendrocyte progenitor cells (OPCs). OPCs form a nonoverlapping grid throughout the adult parenchyma, and they are the most proliferative cell of the adult CNS, providing a ready pool of progenitors to produce new oligodendrocytes and myelin (Hughes et al., 2013). Indeed, myelin turnover from new oligodendrocytes occurs throughout adulthood, and spontaneous remyelination occurs after CNS injury; however, in certain diseases, such as MS, CNS remyelination becomes impaired, contributing to progressive declines in motor function (Franklin and ffrench-Constant, 2008; Young et al., 2013; Hesp et al., 2015).

Although focal demyelinating lesions of CNS white matter are one of the primary causes of function loss in MS, all current treatments for the disease target the immune system, including β-interferons, natalizumab, and teriflunomide (Haghikia et al., 2013). This focus is understandable because MS is thought to be an autoimmune disease and is characterized by overactivation of the immune system, in particular of $CD4^+$ T cells. Nonetheless, these therapies only reduce the relapse rate of MS; they do not prevent progressive functional disabilities or promote CNS repair (Zhang et al., 2013). Only recently has the promotion of OPC differentiation and remyelination become a new focus in MS research. A recently published article in *The Journal of Neuroscience* by Mei et al. (2016) builds upon this trend, identifying κ-opioid receptor (KOR) agonism as a powerful strategy for enhancing oligodendrocyte differentiation and remyelination *in vivo* and providing support for a novel, clinically relevant therapy for MS and other demyelinating diseases.

Mei et al. (2014) used a recently developed micropillar array to simultaneously screen 250 compounds in a GPCR library for the potential to promote OPC differentiation and remyelination. The array features conical "micropillars" that take advantage of the inherent myelinating property of oligodendrocytes, and it allows researchers to visualize the extent of membrane wrapping by oligodendrocytes with 2-photon microscopy and immunolabeling for myelin basic protein (MBP, a marker of mature oligodendrocytes) and PDGFRα (a marker of undifferentiated OPCs). By assessing the extent of MBP^+ myelin "rings" wrapping the micropillars in each well of a plate, molecules can quickly be screened for their potential to alter OPC/oligodendrocyte phenotype.

Using this technique, Mei et al. (2016) found that the selective KOR agonist (±)-U50488 was among the most effective at promoting OPC proliferation, differentiation, and wrapping. The authors further demonstrated that OPCs express KORs and that KOR-null OPCs failed to enhance remyelination following application of (±)-U50488 in culture, confirming that KOR activation in OPCs is critical for promoting remyelination. *In vivo*, KOR deficiency in OPCs delayed myelination during CNS development, although myelination eventually "caught up" to wild-type levels in adulthood. In a lysolecithin model of demyelination targeting the corpus callosum, (±)-U50488 promoted remyelination only in mice possessing KORs on oligodendrocytes. Finally, (±)-U50488 also stimulated

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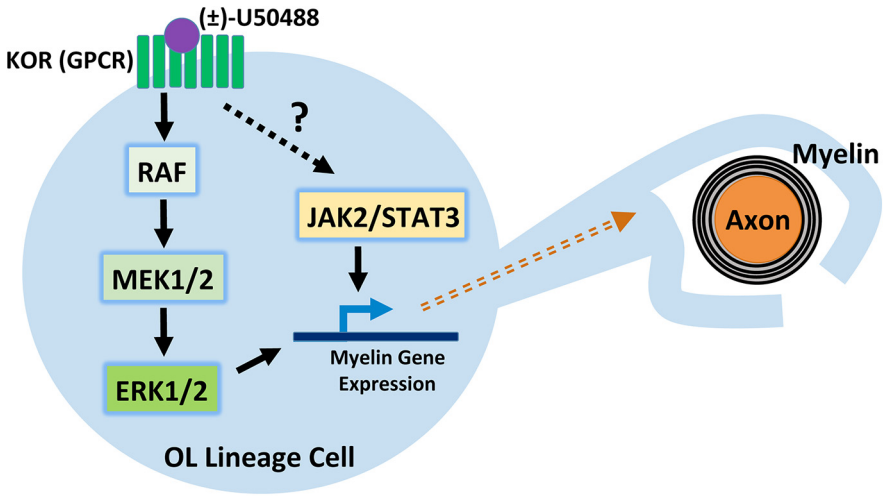

Figure 1. Potential signaling pathways through which KOR agonism could enhance remyelination. KOR binding by agonists, such as (±)-U50488, leads to downstream ERK1/2 activation, CREB phosphorylation, and myelin-related gene (MOG/PLP/MBP) expression. Alternatively, KOR activation could lead to JAK2/STAT3 signaling and pSTAT3-mediated gene transcription.

maturation of human induced pluripotent stem cell-derived oligodendrocytes, providing evidence that the drug has similar effects on human and mouse cells. Together, these findings suggest that KORs are potentially powerful targets to enhance myelination in the CNS.

Other studies have hinted that opioid receptor signaling is important for normal oligodendrocyte function in the developing and injured nervous system. Oligodendrocytes express KORs and μ-opioid receptors, and they produce varying levels of proenkephalin and prodynorphin peptides (ligands for all opioid receptors) during cell maturation (Knapp et al., 2001). Oligodendrocytes from *jimpy* mice, which display deficits in normal myelination due to a point mutation in the proteolipid protein (PLP) gene, have a >90% reduction in KOR expression compared with wild-type mice (Knapp et al., 2009). Additionally, specific KOR antagonism *in vitro* increases oligodendrocyte death and exacerbates glutamate-induced toxicity of mature oligodendrocytes (Knapp et al., 2001). Together, these earlier studies provided indirect evidence for a role of KOR signaling in oligodendrocyte function, although none showed the direct connection between activation of this receptor and myelination.

However, Mei et al. (2016) were not the first to make the connection between KOR agonism and enhanced oligodendrocyte myelination, nor the first to specifically study the effects of (±)-U50488 on oligodendrocyte function. Du et al. (2016) demonstrated the effectiveness of KOR agonism with (±)-U50488 in the treatment of experimental autoimmune encephalomyelitis, a mouse model of MS. Most importantly, Du et al. (2016) showed that KOR-mediated attenuation of disease severity was not due to the actions of KORs on immune cells, but rather through promotion of oligodendrocyte differentiation and remyelination. Moreover, while (±)-U50488 reduced disease severity in wild-type mice, this phenomenon was not apparent in KOR-deficient mice. The present work by Mei et al. (2016) complements these findings and, by using oligodendrocyte-specific KOR knock-out mice (in contrast to the global-KOR knock-out mice used by Du et al., 2016), provides the additional insight that KOR signaling directly affects oligodendrocytes.

One significant question left unanswered by both the Mei et al. (2016) and Du et al. (2016) papers is the potential signaling pathways through which KOR agonists mediate their effects on oligodendrocyte lineage cells. Although no previous research specifically addresses this question, signaling pathways involved in both myelination and KOR activation are likely candidates. KOR signaling results in the activation of specific kinase cascades, including GPCR kinases, the MAPK family (ERK1/2, p38, and JNK), as well as the JAK2/STAT3 and IRF2 cascades (Bruchas and Chavkin, 2010; Finley et al., 2011; Fang et al., 2013). ERK1/2 signaling is the most plausible pathway through which KOR agonism might influence oligodendrocyte myelination, but the STAT3 pathway could also be a mediator of these effects if activated in oligodendrocyte lineage cells by (±)-U50488. We provide evidence suggesting the involvement of these pathways below and in Figure 1.

It is well established that signaling through ERK1/2 is a critical regulator of oligodendrocyte development and myelination. This pathway is required for the maintenance of myelin and axonal integrity in the adult CNS, and ERK1/2 activation in OPCs improves remyelination (Fyffe-Maricich et al., 2013; Ishii et al., 2014). Furthermore, a recent study showed that sustained ERK1/2 activation in preexisting oligodendrocytes of adult mice was sufficient to increase myelin thickness and enhance axon conduction velocity (Jeffries et al., 2016). Importantly, ERK1/2 signaling is necessary for the transcription of key proteins involved in myelination, including MOG, MBP, and PLP, suggesting that KOR activation by molecules, such as (±)-U50488, may enhance myelination through the downstream actions of ERK1/2 (Ishii et al., 2014). Indeed, (±)-U50488 has been shown to activate this signaling cascade in cardiomyocytes (Kim et al., 2011).

An alternative explanation for the remyelinating effects of KOR agonism is the activation of JAK2/STAT3 signaling. Following CNS injury, OPCs increase expression of pSTAT3 in regions of enhanced oligodendrogenesis, and deletion of STAT3 in OPCs reduces oligodendrocyte differentiation during development and after injury (Tripathi and McTigue, 2008; Hesp et al., 2015; Hackett et al., 2016; Steelman et al., 2016). Thus, if KOR agonism specifically triggers STAT3 phosphorylation in oligodendrocyte lineage cells, this could be one avenue through which KORs exert their promyelinating effects, either independently or in syngerism with ERK1/2 signaling.

Understanding downstream KOR activation pathways is crucial for developing selective or partial agonists that can promote remyelination without KOR-related side effects, such as dysphoria or hallucinations, as these are the result of different signaling

cascades downstream of opioid receptor activation (Bruchas and Chavkin, 2010). Salvinorin A, a naturally occurring KOR agonist, which is also readily modifiable, holds promise as a potential starting point for activating specific downstream signaling cascades. For example, a modified version of Salvinorin A, MOM-SAL-B, has been shown to cause only "early phase" ERK1/2 activation following KOR binding (McLennan et al., 2008). This knowledge could be leveraged to engineer partial KOR agonists with specific remyelination-promoting properties.

In conclusion, the work of Mei et al. (2016) and others opens a new door for research on demyelinating diseases, particularly for MS, which lacks targeted remyelination therapies. Although there are several drawbacks to the heavy use of opioid receptor agonists, future research into the mechanism of KOR agonism in oligodendrocyte lineage cells may provide alternative strategies for the development of more clinically appealing pharmacotherapies.

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