# CNS remyelination as a novel reparative approach to neurodegenerative diseases: the role of the purinergic signalling and of the P2Y-like receptor GPR17

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**Abstract**

Oligodendrocytes are the myelin-forming cells in the CNS. They enwrap axons, thus permitting fast impulse transmission and exerting trophic actions on neurons Demyelination accompanied by neurological deficit is a rather frequent condition that is not only associated with multiple sclerosis but has been also recognized in several other neurodegenerative diseases, including brain trauma and stroke, Alzheimer's disease and amyotrophic lateral sclerosis. Recently, alterations of myelin function have been also reported in neuropsychiatric diseases, like depression and autism. Highly relevant for therapeutic purposes, oligodendrocyte precursor cells (OPCs) still persist in the adult brain and spinal cord. These cells are normally rather quiescent, but under specific circumstances, they can be stimulated to undergo differentiation and generate mature myelinating oligodendrocytes. Thus, approaches aimed at restoring myelin integrity and at fostering a correct oligodendrocyte function are now viewed as novel therapeutic opportunities for both neurodegenerative and neuropsychiatric diseases. Both OPCs and mature oligodendrocytes express purinergic receptors. For some of these receptors, expression is restricted at specific differentiation stages, suggesting key roles in OPCs maturation and myelination. Some of these receptors are altered under demyelinating conditions, suggesting that their disregulation may contribute to disease development and could represent adequate new targets for remyelinating therapies.

Here, we shall describe the current literature available on all these receptors, with special emphasis on the P2Y-like GPR17 receptor, that represents one of the most studied receptor subtypes in these cells.

**Keywords**

Purinergic receptors, oligodendrocytes, remyelination.

**Chemical compounds**

2'-Deoxy-$N^6$-methyladenosine 3',5'-bisphosphate ammonium salt (MRS2179); 3-(2-carboxy-4,6-dichloro-indol-3-yl)propionic acid (MDL29,951); 3-[4-[2-[ [6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid (CGS21680); 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH58261); adenosine; ADP; ATP; brilliant blue G (BBG); leukotriene D4 ($LTD_4$); montelukast; N6-cyclohexyladenosine (CHA); oxidized ATP (oxATP); rapamycin; UDP; UDP-glucose.

**Abbreviations**

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ATX, autotaxin; CNS, central nervous system; DRG, dorsal root ganglion; EAE, experimental autoimmune encephalomyelitis; GDNF, glial cell derived neurotrophic factor; GRK, G-protein receptor kinase; MBP, myelin basic protein; MCAo, middle cerebral artery occlusion; MS, multiple sclerosis; mTOR, mammalian target of rapamycin; OPC, oligodendrocyte precursor cell; PDGF, platelet derived growth factor; PVL, periventricular leukomalacia.

## 1. Introduction

The myelin sheath is the fatty insulating layer wrapped around neuronal axons that is essential to increase the speed and efficiency of nerve impulse conduction and maintain axonal integrity. In the CNS, it is produced by specialized glial cells, the oligodendrocytes, which ensheath axons with concentrically multi-lamellar sheets of plasma membrane comprised of specific proteins and lipids (Baumann and Pham-Dinh, 2001).

The formation of myelin is a complex process by which oligodendrocyte progenitors (OPCs) maturate to myelinating cells through phenotypic and morphological changes, reorganization of cytoskeleton, cell polarization and assembly of specialised membrane domains (Bauer et al., 2009; Biname et al., 2013). During myelination, OPCs follow an intrinsic program of proliferation, migration and differentiation through specific developmental stages, which are controlled by exogenous signals, including axonal cues, and the activation of specific intracellular sigalling pathways (Ahrendsen and Macklin, 2013; Gonsalvez et al., 2015; Mitew et al., 2014). Recent review articles have extensively discussed the balance existing among inhibiting and promoting molecules that finally regulates myelin formation, its plasticity and the reciprocal interactions between myelinating glial cells and axons (Emery, 2010; Nave and Werner, 2014).

In this respect, extracellular ATP and uracil nucleotides have been identified as important, activity–dependent axonal signals, that, when non-synaptically released from electrically stimulated axons or from nearby astrocytes, activate purinergic P2 receptors on neighbouring oligodendrocytes (Fields and Burnstock, 2006; Fields and Stevens, 2000). Functional P2X and P2Y receptors (P2XRs and P2YRs), including the P2Y-like receptor GPR17, have been identified on myelinating glial cells at specific developmental stages both *in vitro* and *in vivo* by means of different approaches (Burnstock, 2011, 2015; Fumagalli et al., 2011; Verkhratsky et al., 2009). Of note, the multiple effects of released ATP are not only exerted through purinergic P2 receptors, but also through extracellular ectonucleotidases capable of regulating extracellular ATP, ADP, AMP and adenosine concentrations (Zimmermann et al., 2012). This is particularly important in light of data showing that adenosine is

also crucially involved in modulation of OPC proliferation, migration and myelination (Stevens et al., 2002). The large number of purinergic receptors identified on both OPCs and mature oligodendrocytes, the different signalling pathways modulated by them and the combined activity of ectonucleotide enzymes make this system highly complex.

Here, we shall summarize some crucial aspects of the purinergic regulation of oligodendrocyte functions to shed light on the role of purinergic signalling in myelination. We will also analyze how the purinergic system is dysregulated in diseases characterized by either myelin deterioration or malfunction of myelinating cells. These key issues will be discussed also based on the more recent discovery that myelinating oligodendrocytes do not only electrically insulate axons, but they also exert a trophic effect and contribute to energy supply of myelinated axons by delivering pyruvate/lactate (Funfschilling et al., 2012; Lee et al., 2012; Nave, 2010). This support is essential for normal axonal function *in vivo* and it is of particular relevance for longer axons in which some segments are far away from the neuronal soma, but in close contact to local glial cells (Hirrlinger and Nave, 2014). Axonal metabolic need is also sustained by oligodendrocytes through the release of neurotrophic factors, such as glial cell derived neurotrophic factor (GDNF), and by the presence of gap-junction-forming connexins, that may provide routes for the passage of small metabolites (Wilkins et al., 2003). Of interest, in addition to classical means of cell-to-cell communication, the transfer of vesicles from oligodendrocytes to neuronal endings has been proposed to be a very rapid and efficient means by which these cells may contribute to sustain axonal homeostasis and integrity (Fruhbeis et al., 2013a; Fruhbeis et al., 2013b).

Thus, myelinating oligodendrocytes protect and promote neuronal activity and survival, and the loss of these cells, in diseases such as multiple sclerosis (MS), results in demyelination of axons. Without an intact myelin sheath and glial support, axons become vulnerable to irreversible and cumulative degeneration. It appears clear that strategies aimed at implementing oligodendroglial support and remyelination, namely the myelination of demyelinated axons should result in axon preservation (Franklin and Ffrench-Constant, 2008). In this respect, the discovery that adult parenchymal OPCs

are recruited and proliferate in the demyelinating area has raised the possibility of repairing lesions by implementing the spontaneous endogenous remyelination mediated by these cells (Nishiyama et al., 2009). OPCs, which express the proteoglycan NG2 (and for this reason are also known as NG2-glia) and the platelet-derived growth factor receptor-α (PDGFRα), indeed persist in the adult CNS, constituting the main proliferative cell type of the intact CNS (Dawson et al., 2003). At adult stage, they can be engaged into maturation to sustain a certain degree of basal myelin turnover and plasticity (Young et al., 2013), but they are rapidly mobilized to replace dead oligodendrocytes in demyelinating lesions (Zawadzka et al., 2010), at least at the initial stages of MS.Unfortunately, this initial repair ability is soon lost while disease progresses.

On this basis, we shall start our analysis by discussing a diverse group of diseases, from MS to psychiatric disorders, that have been linked to damage or malfunction of myelinating cells. We shall then discuss why remyelination may represent a novel approach to both neurodegenerative and neuropsychiatric diseases, and how pharmacological interventions could restore adequate conditions to foster endogenous myelination, with a critical focus on the emerging roles of purinergic signalling. Our final aim is to understand if some specific purinoceptor subtype(s) could represent new potential targets for such neuroregenerative approaches.

## 2. Neurological and neuropsychiatric diseases characterized by myelin malfunction

### 2.1. Multiple sclerosis (MS)

This is a typical neurological demyelinating disease where an abnormal autoimmune reaction sustained by both B and T lymphocytes, local microglia and blood-borne macrophages infiltrating the CNS leads to deterioration of myelin sheaths, defects in nerve impulse condition and axonal degeneration (Mallucci et al., 2015). During the past decade, the advent of disease-modifying drugs has substantially changed the work-up of relapsing remitting MS. Besides the typical neurological

dysfunctions associated with the disease, defects in myelin insulation can lead to impaired cognitive function in 40% of MS patients (Kujala et al., 1997).

Early treatment initiation is now recommended to maximise the efficacy of the currently available therapies, which mostly consist of immunomodulating and immunosuppressive strategies acting against the inflammatory and immune-mediated components of the disease. By contrast, treatment of the progressive phase of MS, which is characterised by the steady accumulation of irreversible disability, is by far sub-optimal (Rovaris et al., 2006). A wide range of immunosuppressants (including methotrexate, cladribine, cyclophosphamide, methylprednisolone, azathioprine, cyclosporine, and mitoxantrone) have been used in patients with secondary progressive MS. In general, modest benefits in delaying time to onset of sustained progression of disability have been reported (with even transient exacerbation of neurological symptoms in some cases), together with major toxic effects due to non-selective immunosuppression, that significantly restricts the use of these drugs in these patients. The observed failure of these treatments suggests that, once the cascade of events leading to neuronal and axonal loss in secondary progressive MS is established, even an effective suppression of inflammation does not protect from clinical disease progression. Stimulation of undifferentiated oligodendrocyte precursor cells that are often observed in the areas of chronic demyelination in MS patients could result in repair of demyelinated lesions and axonal preservation.

### 2.2. Ischemia

Stroke is a disease of cardiovascular origin that results from an interruption of the blood supply to a specific area of the brain, usually caused by the occlusion of a cerebral artery either by an embolus or by local thrombosis. It is currently acknowledged that an ideal therapeutic intervention to decrease stroke-associated disability should comprise both neuroprotective and neurorestorative approaches implementing local spontaneous post-injury repair mechanisms. However, spontaneous repair is very

limited and constrained with poor improvement of neurological outcome (Benowitz and Carmichael, 2010).

Data accumulated so far provide evidence that not only neurons but also oligodendrocytes are vulnerable to ischemia (Dewar et al., 2003). Loss of these cells impairs axonal integrity contributing to stroke-associated deficits to a greater extent than what was believed in the past.

Fostering endogenous myelin repair has recently emerged as a relatively unexploited new therapeutic approach to cerebral ischemia. Lessons from classical demyelinating diseases like MS indeed suggest that: (i) besides enabling fast impulse transmission, oligodendrocytes sustain axonal integrity and neuronal survival and, (ii) reconstruction of damaged myelin results in axonal function restoration and more complete functional recovery.

Also in stroke, the discovery that parenchymal OPCs are recruited and proliferate in the peripheral area of the ischemic "core" (called the "penumbra") (Zhang et al., 2013) has raised the possibility of repairing ischemic lesions by implementing the spontaneous endogenous remyelination mediated by these cells. Interestingly, in the first two weeks after ischemia, OPCs accumulate in the peri-infarct area and, within two months, they differentiate into mature oligodendrocytes expressing the major component of myelin (i.e. myelin basic protein, MBP) (Zhang et al., 2011; Zhang et al., 2012). These findings suggest that the manipulation of OPCs might be a good therapeutic strategy to strengthen the endogenous mechanisms of remyelination and repair in ischemic brain.

### 2.3. Amyotrophic Lateral Sclerosis (ALS)

Therapeutic approaches to amyotrophic lateral sclerosis (ALS) have been so far aimed at retarding degeneration of spinal cord motor neurons by direct neuroprotective strategies or by reducing astrocyte/microglial reactions (O'Connor and Boulis, 2012). New insights in ALS come from data on resident spinal cord OPCs in the mutant hSOD1 G93A mouse model. Specifically, besides motor neurons, oligodendrocytes also undergo very early death in ALS. Moreover, at advanced disease stage, OPCs exhibit active proliferation and accelerated differentiation into mature

oligodendrocytes, suggesting a remyelination attempt, that is, however, largely unsuccessful (Kang et al., 2013). Also here, in a similar way to stroke, it has been increasingly recognized that restoration of oligodendrocyte function and early prevention of myelin deterioration results in axonal preservation and more benign disease progression, slowing down motor neuron degeneration, helping reconstruct myelin sheaths, re-establishing neuron-to-neuron communication, retarding disease progression and increasing patients' lifespan.

In particular for ALS, focus has been recently drawn to implementation of oligodendroglial metabolic support to neurons with pharmacological agents. Oligodendroglial transfer of glycolytic intermediates to neurons through the monocarboxylic transporter MCT1 has been found to be impaired in mSOD1 mice, concomitant with oligodendrocyte degeneration and myelin loss (Kang et al., 2013). Downregulation or inhibition of MCT1 resulted in axonal degeneration, emphasizing the importance of lactate for neurons (Lee et al., 2012). Thus, therapeutic approaches enhancing metabolic energy supply to neurons may be helpful for preventing motor neuron degeneration in ALS.

### 2.4. Alzheimer's disease (AD)

Myelin loss, reduction in size of corpus callous, oligodendrocyte cell death are some features of white matter alterations that are frequently observed in human Alzheimer'disease (AD) (Bartzokis et al., 2003; Ihara et al., 2010; Roher et al., 2002; Svennerholm and Gottfries, 1994).

Interestingly, in both rodent models and patients, major pathological hallmarks of AD, like β-amyloid (βA) plaques, are also accompanied by focal demyelination in grey matter (Mitew et al., 2010). Myelin defects are found in both sporadic and preclinical AD cases and are absent in plaque-free cortical regions, suggesting a specific association with disease development (Mitew et al., 2010). Of note, dystrophic plaque-associated neurites are also demyelinated, suggesting that, in the cortical grey matter, this event is linked to the aberrant axonal sprouting underlying dystrophic neurite formation and impairing cortical processing (Mitew et al., 2010). Thus, also for this pathology, novel therapeutic approaches to AD could aim at implementing the differentiating and remyelinating capabilities of

OPCs, instead of directly targeting dystrophic and dying neurons (Behrendt et al., 2013; Nielsen et al., 2013).

### 2.5. Psychiatric disorders

Of note, it has been recently proposed that abnormal myelin formation is suspected of contributing to several mental illnesses, including depression, schizophrenia, autism, bipolar disorders and dyslexia (Fields, 2008). Myelination changes in prefrontal cortex result from social isolation during critical periods in mouse development. Similarly, in adult mice, social isolation results in epigenetic changes in oligodendrocytes that impair myelination in prefrontal cortex (Liu et al., 2012; Makinodan et al., 2012). Together with new data showing that white matter structure is dynamic and myelin can be regulated by impulse activity, such findings strongly suggest that experience influences myelin formation, which, in turn, supports learning and improvement of skills (Fields, 2008).

Importantly, myelination continues into adulthood in white matter tracts of association areas such as frontal cortex, which undergoes extensive myelination after 20 years of age (Lebel et al., 2012) and affects information processing by regulating the velocity and synchrony of impulse conduction between distant cortical regions. Cognitive decline in aging also parallels subtle changes in the integrity of white matter (Gootjes et al., 2004). Impaired cognitive ability, mood disorders or hallucinations, disorganized thinking and accompanying psychiatric illness may result from slowed or desynchronized impulse conduction between distant cortical regions (Fields, 2008). Globally, these findings suggest that neuropsychiatric diseases may take advantage of therapies aimed at restoring myelin integrity.

## 3. Purinergic regulation of oligodendrocyte function and myelination

As mentioned above, under normal physiological conditions, ATP is released from neurons and can then act on oligodendrocytes by activating P2 or (upon degradation to adenosine), P1

receptors. Importantly, ATP can be also released by both astrocytes and microglial cells, that thus also contribute to activating purinergic receptors on oligodendrocytes (Fields and Stevens, 2000). After acute CNS injury, all these cells release ATP acting as a diffusible danger signal to alert responses to damage and promote repair. However, excessive extracellular ATP release also resulting from cell breakdown and death can contribute to neurodegeneration (Di Virgilio et al., 2009). Here, we shall summarize the roles of P1 and P2 receptors in oligodendrocyte pathophysiology. A summary of the various P1 and P2 receptors expressed by both OPCs and oligodendrocytes at different differentiation stages is presented in Table 1.

### 3.1. Adenosine receptors and opposite roles of adenosine in oligodendrocyte maturation

Adenosine has a key role in different OPC processes such as migration, proliferation and maturation (Coppi et al., 2015; Coppi et al., 2013a; Stevens and Fields, 2000; Stevens et al., 2002). In the CNS, adenosine, presumably generated by the hydrolysis of extracellular ATP released from axons, can both promote and impair myelination, and this is largely due to the activation of different adenosine receptors expressed on oligodendrocytes at different stages of maturation (Fredholm et al., 2011; Othman et al., 2003; Stevens et al., 2002) (Table 1). It has been proposed that adenosine, through the activation of the $A_1$ receptors, is the first myelination trigger thanks to its enhancement of inward $K^+$ ($I_K$) currents (Stevens et al., 2002). Through the $A_1$ receptor, adenosine exerts a concentration-dependent reduction of OPC proliferation in the presence of the mitogen PDGF and promotes their differentiation toward pre-myelinating oligodendrocytes. The $A_1$ receptor also intervenes at more advanced differentiated stages when it also promotes axonal myelination in DRG/OPC co-cultures (Stevens et al., 2002)(Table 1). In line with these findings, the $A_1$ receptor agonist N6-cyclohexyladenosine (CHA) did not only exert a protective effect on lysolecithin induced demyelination of rat optic nerve, but also induced remyelination (Asghari et al., 2013).

Conversely, the selective $A_{2A}$ receptor agonist CGS21680 significantly reduced the number of mature oligodendrocytes, whereas the number of early OPCs expressing the proteoglycan NG2

was increased (Coppi et al., 2013a) (Table 1). Interestingly, neither cell viability nor proliferation were affected, suggesting that receptor activation blocks cells at a more undifferentiated stage. In the middle cerebral artery occlusion (MCAo), a rodent model of brain ischemia, the selective $A_{2A}$ antagonist SCH58261 prevented myelin disorganization in basal nuclei, and reduced brain damage, microglial activation, and neurological deficits (Melani et al., 2009). *In vivo* blockade of the $A_{2A}$ receptor also prevented the onset of experimental autoimmune encephalomyelitis (EAE), a rodent model of MS (Mills et al., 2012). Upregulation of the $A_{2A}$ receptor was observed in the white matter of patients with secondary progressive MS, and the density of this receptor positively correlated with worsening of disability in patients (Rissanen et al., 2013), suggesting that aberrant $A_{2A}$ receptor regulation may participate to the onset of demyelination and/or to impaired myelin repair.

The opposite effects mediated by $A_1$ and $A_{2A}$ receptors on oligodendrocyte differentiation and myelination are at least partly due to their different coupling to cAMP levels and their different ability to elicit $K^+$ currents. In early OPCs, most currents are carried out by outward rectifying channels. However, during cell differentiation, a gradual decrease of outward $K^+$ currents and a parallel increase in $I_K$ conductance in mature oligodendrocytes is observed (Coppi et al., 2015; Coppi et al., 2013a). While, the activation of $A_1$ receptor lowers adenylyl-cyclase activity and promotes the generation of $I_K$ currents, the $A_{2A}$ receptor is coupled to increases of intracellular cAMP and inhibition of $I_K$ currents, thus opposing cell maturation (Coppi et al., 2013a).

### 3.2. P2X7 receptor as a potential sensor of myelin preservation

The P2X7R has been described as an emerging target in CNS diseases in glial cells (Sperlagh and Illes, 2014), but its role in oligodendrocytes has been poorly studied. According to intracellular $Ca^{2+}$ measurement and whole cell recording, despite the presence of several different P2X receptors in OPCs (Table 1), P2X7R is the only P2X receptor functionally active in these cells (Agresti et al., 2005a). Experimental evidence shows that oligodendrocytes and their progenitors express P2X7Rs *in vitro* (Matute et al., 2007; Wang et al., 2009). However, the functional role of this receptor in the

physiology of oligodendrocytes is still unclear. Mature oligodendrocytes express functional P2X7Rs ~~receptors~~ mediating rapid increases in $[Ca^{2+}]_i$, pore formation in cell membrane and cell death *in vitro* and *in vivo*. P2X7R is located mainly on oligodendrocytes, in myelin sheath, but not in axons. In optic nerves subjected to oxygen/glucose deprivation, myelin was focally disrupted in many axons. In this *in vitro* model P2X7R pharmacological blockade, via the irreversible non-competitive antagonist oxATP, limited $Ca^{2+}$ flux and significantly reduced myelin damage (Domercq et al., 2010). A similar result was obtained by either removing extracellular $Ca^{2+}$ or in the presence of brilliant blue G (BBG), suggesting that, during ischemia, the extracellular concentrations of ATP rise to levels sufficient to activate P2X7Rs (Domercq et al., 2010; Matute, 2008).

The expression of P2X7R in myelin sheaths may be relevant to myelin formation and preservation, and may act by sensing electrical activity in the axons (Matute, 2008). P2X7R receptor expression is increased in the normal-appearing optic nerve of MS patients before lesion formation (Matute et al., 2007); consequently, also P2X7R signalling is enhanced in these regions making oligodendrocytes more vulnerable to ATP excitotoxicity and thus contributing to the onset and/or progression of the early phases of the disease (Matute, 2008). In line with this hypothesis, P2X7R blockade ameliorates chronic Experimental Autoimmune Encephalomyelitis (EAE) by reducing demyelination, delaying its progression and thus improving axonal conduction (Grygorowicz et al., 2011; Matute et al., 2007). However, the relevance of this finding to the etiology of MS is not known.

### 3.3. P2Y receptors and OPC differentiation

Although several P2YRs have been found to be expressed in oligodendrocytes (Table 1), little is known about their functional roles in OPC differentiation, maturation and myelination.

ATP was able to promote the migration of OPCs in a dose-dependent manner up to 75% over control after only 5 hours of incubation, and was more effective than PDGF, a classic chemoattractant for these cells (Agresti et al., 2005b). ADP, but not UTP or BzATP, induced a similar effect, and this was inhibited by the pre-incubation with the $P2Y_1R$ antagonist MRS2179, suggesting that $P2Y_1R$ is

the main purinergic player in OPC chemotaxis. Both in purified OPCs and in cerebellar slices, treatment with ADPβS induced significant maturation of oligodendrocytes, whereas effect on proliferation was only marginal (Agresti et al., 2005b). Of note, $P2Y_1R$ and $A_1R$ can form hetero-dimers, which may reciprocally influence their pharmacology and coupling to transduction systems (Yoshioka et al., 2002). However, whether the formation of such heteromers affects OPCs and oligodendroglial function is completely unknown. Moreover, ATP and its hydrolysis products ADP and adenosine could act in concert and differently contribute to OPC proliferation or differentiation (see also above).

A recent analysis revealed that adult OPCs have a transcriptome more similar to that of oligodendrocytes than to neonatal OPCs, but revert to a neonatal-like transcriptome when activated after cuprizone-induced demyelination in brain (Moyon et al., 2015). Interestingly, activated OPCs showed enhanced differentiation and migration capabilities, in parallel to increased expression of several genes including IL-1β, the chemokine MCP-1 and the $P2Y_2R$ (Table 1). However, the functional role of $P2Y_2R$ in this model has not been investigated (Moyon et al., 2015).

Expression of $P2Y_{12}R$ has been described *in vivo* in oligodendrocytes positive for CNPase or the myelin basic protein MBP of cerebral cortex, subcortical areas and periventricular white matter, mostly in the paranodal regions of the fibers. This localization was confirmed in the corticospinal tract suggesting highly conserved tissue homogeneity and a potential role in myelination (Amadio et al., 2006).

In post-mortem MS brains, $P2Y_{12}R$ immunoreactivity in proximity to the lesions directly correlated with the extent of demyelination found in gray matter cortical plaques and subcortical white matter, suggesting that loss of this receptor might be detrimental to tissue integrity (Amadio et al., 2010).

$P2Y_{12}R$ apparently plays a critical role in the last phases of OPC differentiation, in part interacting with autotaxin (ATX), an extracellular matrix protein. When active, ATX signalling promotes the typical shape change of immature oligodendrocytes, with a strong expansion in process network. In OPCs, silencing of $P2Y_{12}R$ significantly impaired the morphological changes associated

with oligodendroglial differentiation. Interestingly, in primary cultures, $P2Y_{12}R$ is only expressed in immature oligodendrocytes (positive for O4) and in early precursors, validating a role for the ATX-$P2Y_{12}$ system specifically at late OPC differentiation stages (Dennis et al., 2012). Moreover, CHO-K1 cells stably transfected with $P2Y_{12}R$ acquire a state of intermediate adhesion similar to that observed in OPCs. To confirm the specificity of this signalling, only in transfected cells was treatment with the $P2Y_{12}R$ agonist ADP able to inhibit cAMP accumulation. Focal adhesion mediated by $P2Y_{12}R$ is triggered by a physical interaction between the receptor and ATX, and is mediated by the GTPase Rac1 (Dennis et al., 2012).

### 3.4. The P2Y-like receptor GPR17 in physiological oligodendroglial functions and myelination

Originally identified as an incomplete sequence of a human GPCR many years ago (Blasius et al., 1998), GPR17, a Gi coupled receptor inhibiting the formation of cAMP, has been an orphan receptor for a long time. The GPR17 sequence had been initially described as the result of a cloning strategy based on the use of RT-PCR degenerate oligonucleotide primers designed on the sequences of the $P2Y_1$ and $P2Y_2$ receptors, with the final aim of identifying new members of this receptor family (Blasius et al., 1998). GPR17 was later found to be at an intermediate structural and phylogenetic position between already known P2Y and CysLT receptors, and GPR99 (recently proposed as the third CysLT receptor (Kanaoka et al., 2013), in the so called "purine receptor cluster" of class A GPCRs.

GPR17 displays the typical 7-transmembrane (TM) domain topology of GPCRs, with an amino acid identity with the known P2Y and CysLT receptors between 21-48% (Abbracchio et al., 2006; Lecca et al., 2008). All these receptors show partial or complete conservation of a H-X-X-R/K amino acid motif in TM6 (and also of a K-E-X-X-L motif in TM7, in the case of $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) that are important for ligand recognition and have been proposed to represent specific molecular signatures for these receptors (Lecca et al., 2008). Homology Modelling studies combined with other *in silico* tools have been performed to raise hypothesis on the molecular interaction between the short isoform

of GPR17 and its putative endogenous ligands (Calleri et al., 2010; Parravicini et al., 2010; Parravicini et al., 2008), as well as to identify new potential ligands (Eberini et al., 2011). In both P2Y, CysLT1 and CysLT2 receptors, ligand binding is critically dependent on the basic Arginine residue belonging to the conserved TM6 motif (Abbracchio et al., 2006). Computational studies and steered molecular dynamics simulations on the mutated R255I GPR17 receptor suggested that this also holds true for Arg255 of GPR17 (Calleri et al., 2010; Parravicini et al., 2010; Parravicini et al., 2008).

GPR17 has been demonstrated to respond to UDP, UDP-glucose and UDP-galactose in the micromolar range (Benned-Jensen and Rosenkilde, 2010; Ciana et al., 2006; Daniele et al., 2014; Lecca et al., 2008) fully consistent with the concentrations at which these endogenous ligands activated their already known cognate P2Y receptors (Abbracchio et al., 2006). Interestingly, GPR17 is also activated by cysteinyl-leukotrienes ($LTC_4$, $LTD_4$ and $LTE_4$), inflammatory mediators that are not chemically or structurally related with nucleotides. Functional interactions between "classical" P2Y and CysLT receptors had been already described (Mamedova et al., 2005; Paruchuri et al., 2009). More recently, GPR17 has been reported to also respond to emergency signals like oxysterols, in a similar way to other related receptors involved in inflammatory responses, like Epstein Barr virus induced gene receptor-2 and CXCR2 (Sensi et al., 2014). Moreover, a high-throughput virtual screening of GPR17 binding site with more than 130,000 lead-like compounds, followed by in vitro pharmacological validation of the top-scoring chemical structures also allowed the identification of five agonists or partial agonists with very diverse chemical structure (Eberini et al., 2011). Globally, these data suggest that GPR17 may promiscuously respond to different signalling molecules depending on specific pathophysiological conditions and emergency situations. GPR17 responses may also vary depending upon its heterodimerization with other receptors, including P2Y and Cys-LT receptors (Maekawa et al., 2009), which could help explain why agonists of GPR17 have such diverse chemical structures. This also suggests that GPR17 may function as an adaptor protein for enhancing the agonist repertoire of other GPCRs, consistent with the conclusion that MDL29,951, an agonist of GPR17, can engage $G_q$ as well as $G_i$ proteins (see also below).

A few years after its initial characterization in recombinant systems, GPR17 was reported to be mainly expressed in the CNS, in particular on OPCs (Boda et al., 2011; Chen et al., 2009; Fumagalli et al., 2011; Lecca et al., 2008). Since then, much interest on GPR17 has been aimed at understanding its function in CNS myelination. *In vitro* studies on purified rat postnatal OPC cultures showed that GPR17 expression labels a specific temporal window of the oligodendroglial differentiation process. It starts to be expressed in early precursors positive for the proteoglycan NG2 and PDGFRα, with an expression peak in still immature oligodendrocytes that also express O4, but is then progressively down-regulated when oligodendrocytes start expressing myelin markers such as myelin basic protein (MBP) (Fumagalli et al., 2011). Based on this strict expression time-frame, GPR17 is now widely accepted as a new marker of an intermediate phase of OPC differentiation (Crociara et al., 2013; Mitew et al., 2014; Nakatani et al., 2013).

In cultured rat OPCs, early GPR17 silencing with small interfering RNAs profoundly affected their ability to generate mature oligodendrocytes, suggesting that cells are retained at a less differentiated stage and do not progress to maturation in the absence of this receptor (Fumagalli et al., 2011). However, interference with GPR17 physiological downregulation at late stages of OPC differentiation by transfection of a reporter plasmid for GPR17 overexpression also maintained cells at a more immature phenotype, since they never expressed the mature marker CNPase (Fumagalli et al., 2015). In line with this result, CNP-GPR17 transgenic mice aberrantly and un-timely expressing GPR17 in late stage oligodendrocytes that are already positive for CNPase showed defective myelinogenesis, motor disabilities, tremors and precocious death within the second week of life, likely due to a disrupted regulation of terminal maturation (Chen et al., 2009).

Thus, interference with GPR17 stage-restricted expression resulting in un-programmed receptor expression in late OPCs completely alters the differentiation program of these cells. As a potential mechanism at the basis of this effect, it has been demonstrated that, when GPR17 is expressed, the differentiation inhibitors ID2 and ID4 abundantly translocate into the nucleus, thus preventing Olig1 from binding to DNA and inhibiting oligodendroglial maturation (Chen et al.,

2009). On the other hand, the GPR17 promoter is efficiently activated by medium conditioned by cortical neurons, suggesting that extrinsic signals including molecules released from neurons can turn on the GPR17 gene (Fratangeli et al., 2013). Globally, these findings suggest that GPR17 exerts opposite stage-specific roles: a positive role for differentiation in early OPCs and a negative function for oligodendroglial maturation in late OPCs. They also suggest that, in late OPCs, physiological GPR17 silencing is needed to allow cells to complete their maturation program.

Mice deficient for GPR17 have increased and more precocious myelination, that has been interpreted as evidence that GPR17 may negatively regulate the whole process (Chen et al., 2009). However, in these GPR17$^{-/-}$ mice, the myelin g-ratio and the total number of oligodendrocytes were comparable to those of control mice, suggesting that other myelin regulators take over the loss of GPR17 and activate compensatory mechanisms as a result of constitutive embryonic GPR17 knock out. For this reason, while potentially interesting for neuropsychiatric diseases like autism, where accelerated white matter maturation has been shown (Ben Bashat et al., 2007; Weinstein et al., 2011), constitutive GPR17$^{-/-}$ mice do not seem to represent an appropriate model to study GPR17 regulation of myelination under physiological conditions or neurological dysfunction occurring during adulthood.

### 3.4.1. Pharmacology and signalling of GPR17

As already mentioned, activation of early OPCs with UDP-glucose promoted the conversion to more mature cells expressing myelin markers (Ciana et al., 2006). Consistent with these data, GPR17 antagonists like Cangrelor delayed the ability to generate mature cells (Fumagalli et al., 2011; Lecca et al., 2008), suggesting that GPR17 endogenous ligands are basally released in culture and are responsible for the observed spontaneous *in vitro* maturation of these cells. In another independent study, while not modifying the potential of adult multipotent neural stem cells, Montelukast, which also acts as a GPR17 antagonist (Ciana et al., 2006; Fratangeli et al., 2013; Lecca et al., 2008; Pugliese et al., 2009), markedly increased their proliferation rate, supporting retention of cells at a more

undifferentiated stage (Huber et al., 2011). In line with these findings, treatment of highly proliferating neurospheres from murine oligodendroglioma cells with UDP-glucose, UDP or $LTD_4$ reduced proliferation and expanded the pool of $Olig2^+$ oligodendrocytes, suggesting that GPR17 activation directs cells to differentiation (Dougherty et al., 2012).

Based on these findings and on the proposed role of cAMP in cell differentiation (Malone et al., 2013), it can be hypothesized that, in early OPCs, by directly inhibiting intracellular cAMP levels via Gi (see above), GPR17 agonists keep cells at an immature state necessary to prepare them for myelination. At later stages, when OPCs exit from the cell cycle and commit themselves to terminal differentiation, GPR17 undergoes downregulation and internalization, to allow cells to resume the appropriate cAMP levels necessary for maturation. GPR17 phosphorylation by G-protein receptor kinases (GRKs), followed, sequentially, by receptor desensitization, removal from the membrane and internalization has been proposed as a potential mechanism at the basis of GPR17 physiological silencing in late OPCs (Daniele et al., 2014; Fratangeli et al., 2013). A cross talk between GRKs, the mammalian target of rapamycin mTOR and the ubiquitin-protein ligase MDM2 has been more recently demonstrated to specifically regulate the availability of GPR17 at the plasma membrane (Fumagalli et al., 2015).

Besides cAMP inhibition, GPR17 has been also shown to specifically mediate activation of delayed rectifier $K^+$ currents in a sub-population of OPCs and pre-immature $O4^+$ oligodendrocytes, but not in mature oligodendrocytes. This effect was shown to contribute to the terminal maturation of OPCs and to their migratory abilities. The transient nature of this effect was fully consistent with the expression pattern of GPR17, and the $K^+$ currents themselves were shown to gradually disappear along with GPR17 physiological downregulation in late OPCs (Coppi et al., 2013b). These findings suggest that these currents also correlate with the GPR17-mediated facilitating effect in OPC maturation. However, how this additional signalling pathway intersects with the cAMP production system in OPCs still remains to be determined.

In contrast with the above studies, in a more recent *in vitro* study, the new postulated synthetic

GPR17 agonist ligand MDL29,951 inhibits, rather than stimulates, oligodendrocyte maturation (Hennen et al., 2013). At variance from the previous literature showing a primary coupling with Gi-proteins, in a variety of different heterologous expression systems, MDL29,951-stimulated GPR17 engaged the entire set of intracellular adaptor proteins for GPCRs. In OPCs, MDL29,951 rapidly mobilized intracellular $Ca^{2+}$ in a concentration-dependent manner and engaged both Gi and Gq. Despite this demonstrated promiscuity in heterologous expression systems, it however seems that in native systems GPR17 mainly couples to Gi.

It is worth noting that MDL29,951 is not selective for GPR17, but has been originally developed as a ligand for the glycinergic site of the glutamate NMDA receptor, at which it shows very specific interaction in the nM range (Salituro et al., 1992). OPCs do express NMDA receptors and their knock down by specific silencing RNAs or by glutamate receptor antagonists resulted in a marked block of OPC maturation (Li et al., 2013). By acting at the glycinergic site of the NMDA receptor, MDL29,951 could markedly interfere with OPC differentiation, thus inhibiting myelination, independently of its action on GPR17.

### 3.4.2. GPR17 as a sensor of damage in disease conditions

During neuroinflammation and upon trauma and ischemia, nucleotides are massively released from activated cells or leak from injured or dead resident cells (Cieslak et al., 2011). The synthesis of cysLTs is also augmented under inflammatory conditions (e.g. in MS plaques and in the spinal cord of EAE mice) (Whitney et al., 2001). Thus, under such conditions, both nucleotides and cysLTs are likely to activate GPR17.

While physiologically GPR17 is mostly an oligodendroglial receptor, within 48 h after acute injury, GPR17 is induced, sequentially, in dying neurons inside and at the borders of the ischemic/traumatic lesion, in infiltrating microglia/macrophages and in activated parenchymal OPCs in the lesion's surrounding areas, with similar expression patterns in different models of disease (Boda et al., 2011; Ceruti et al., 2009; Ciana et al., 2006; Lecca et al., 2008; Zhao et al., 2012)(Figure 1).

Seventy-two h after MCAo, in the regions surrounding the ischemic area and in the ipsilateral corpus striatum, a higher number of GPR17-expressing OPCs was found compared to contralateral hemisphere (Lecca et al., 2008), suggesting OPC recruitment and/or increased proliferation in response to demyelination. In the same model, administration of GPR17 antagonists, such as Cangrelor or Montelukast, or GPR17 silencing due to the *in vivo* delivery of specific antisense oligonucleotides during acute injury resulted in significant reduction of infarct ischemic volume (Ciana et al., 2006; Lecca et al., 2008). In contrast, in a rat neonatal model of ischemic periventricular leukomalacia (PVL), a common cerebral white matter injury, a GPR17 agonist (UDP-glucose) significantly contributed to the recovery of myelin sheaths and improved motor functions, learning and coordination in PVL pups (Mao et al., 2012). This discrepancy may be due to the different outcome of the ischemic insult in neonatal brain compared to adults, and in the different availability of newborn OPCs.

Dysregulated expression of GPR17 has been described also in human samples from patients with traumatic brain injury (Franke et al., 2013). In both neurosurgical and autopsy specimens, GPR17 expression was evident inside the contused core and progressively declined distally according to a spatio-temporal gradient. Inside and around the core, GPR17 labeled dying neurons, reactive astrocytes, and activated microglia/macrophages. In peri-contused parenchyma, GPR17 decorated OPCs, some of which had proliferated as demonstrated by Ki67 labelling of nuclei, indicating remyelination attempts. In agreement with the above data, in a double transgenic model of Alzheimer's disease (the APPPS1 mouse), GPR17-positive cells accumulate close to Aβ plaques in gray matter, revealing receptor upregulation as a feature of oligodendroglial reactivity also in this pathological condition (Boda et al., 2011). In a similar way, acute damage to myelin induced by lysolecithin injection in corpus callosum induced a strong overexpression of GPR17 at the lesion site 10 days after injury (Boda et al., 2011). Similar GPR17 changes have been reported also in human MS, a typically demyelinating disease in which remyelination does occur after the initial myelin damage but fails after multiple episodes of demyelination, which leads to axonal degeneration and

progressive disability (Franklin and Ffrench-Constant, 2008). Increased concentrations of the GPR17 transcript were reported in MS plaques as compared with white matter from non-neurological donor samples and normal-appearing white matter from MS donors (Chen et al., 2009). A similar increase was found in EAE mice (Chen et al., 2009). Of note, Montelukast, an antagonist at both CysLT1 and GPR17, attenuated CNS infiltration of inflammatory cells and the clinical symptoms of EAE (Wang et al., 2011).

Thus, GPR17 is abnormally upregulated in neurodegenerative conditions characterized by myelin disruption, independently of the original cause. Based on the current knowledge on GPR17 under physiological conditions, it could be hypothesized that, after damage, GPR17 is initially induced to promote remyelination and repair; however, at later stages, due to lack of appropriate environmental stimuli, presence of inflammatory signals and/or intrinsic factors, the physiological downregulation of GPR17 is impeded, thus freezing cells at an immature stand-by stage, where they are neither proliferating, nor differentiating. Interventions targeting GPR17 may help to bypass this checkpoint, accomplishing terminal maturation and promoting neurorepair. In this respect, GPR17 is an ideal target, since it is a membrane receptor that, at variance from other intrinsic regulators of oligodendrogenesis, can be easily targeted and manipulated with pharmacological agents.

## 4. Conclusions

The literature data summarized here show that demyelination is not exclusively associated with multiple sclerosis but represents a common feature of several neurodegenerative conditions, such as those associated with stroke, brain trauma, Alzheimer's disease and ALS. Moreover, evidence is accumulating to suggest that myelin dysfunction also contributes to some neuropsychiatric diseases. Remyelination or restoration of a correct myelin function does not only re-establish fast nerve impulse transmission in the CNS and foster neurological recovery after injury, but also prevents axonal damage and neurodegeneration, thanks to the metabolic, energetic and trophic support exerted by myelinating oligodendrocytes on axons.

Both OPCs and maturing oligodendrocytes express several P1 and P2 purinoceptor subtypes. The expression of some of these receptors is strictly dependent on specific OPC differentiation stages, suggesting that there may be a timely and coordinated interplay between different receptors in orchestrating the progression of cells to myelinating phenotypes. In this respect, the putative relationship between GPR17 and $P2Y_{12}$ seems particularly relevant, since the latter is upregulated in immature oligodendrocytes exactly when GPR17 undergoes downregulation, suggesting a role for GPR17 in initiating differentiation and in preparing cells to myelination, and a role for $P2Y_{12}$ in completing the process. Elucidation of the timely sequential involvement of the two receptors during OPC differentiation may help set up combined pharmacological approaches aimed at a better and more integrated neuroregenerating approach.

The role of several other purinoceptors found on either OPCs and/or mature oligodendrocytes is less known. Evidence is accumulating for opposite roles for the $A_1R$ and $A_{2A}R$ in OPC differentiation and myelination, but it is presently unknown whether and how the pathways activated by these receptors intersect with those regulated by GPR17 and $P2Y_{12}R$. Several key issues remain obscure. For example, are different purinoceptors recruited under different pathophysiological conditions? Are their activated pathways redundant? How are their expression and function altered under disease conditions? Despite some available data on the up- or downregulation of specific receptor subtypes and on the de novo induction, under demyelinating conditions, of purinoceptors that are not normally expressed by adult OPCs, how all these events are coordinated still remains to be established. Addressing all these issues in dynamic conditions reproducing the events occurring during disease development will be essential in years to come to identify the correct pharmacological approach to develop purinergic agents of potential utility for new neuroregenerative therapies.

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**Conflict of interest**

The authors declare no conflict of interest.

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**Figure legends**

**Figure 1. Upregulation of GPR17 in mouse models of CNS degeneration.**

The number of GPR17-positive cells was found to be upregulated in brain cortex at the borders of ischemic lesions 48h after occlusion of middle cerebral artery (MCAo) compared to the contralateral hemisphere (Con) (A). A similar increase was found after both a cortical stab wound (SW) and injection of lysolecithin (Lys) in the corpus callosum in two additional models of acute lesions (B and C) compared to controls (Ctr). An increase in the number of GPR17-positive cells was also found around β-amyloid deposits (βA in red fluorescence) in the cerebral cortex of mice carrying a double mutation typical of Alzheimer's disease (APPPS1) compared to WT animals (D). *In situ* hybridization showed GPR17 upregulation in spinal cord oligodendrocytes of mice in which experimental autoimmune encephalomyelitis (EAE) has been induced (E). Scale bar in A, B, C and D 50 µm; 200 µm in E. Modified from Boda et al., 2011. Panel E is adapted by permission from Macmillan Publishers Ltd: Nature Neuroscience Chen et al., 12(11):1398-406, copyright 2009.

**Table 1**: **Role of P1, P2 receptors and of the P2Y-like receptor GPR17 in oligodendrocyte differentiation and function.**

| Receptor | Coupling | Differentiation stage | Functional roles | Pathological alterations | References |
|---|---|---|---|---|---|
| $A_1$ | Gi | All the stages | Promotes OPC differentiation to pre-oligodendrocytes and myelination of axons in OPC/DRG co-cultures; promotes cell migration; inhibits proliferation | N.A. | Stevens et al., 2002; Othman et al., 2003 |
| $A_{2A}$ | Gs; inhibits $I_K$ currents | All the stages | Inhibition of OPC differentiation | Upregulated in MS | Coppi et al., 2013a |
| $A_{2B}$ | Gs | OPCs | N.A. | N.A. | Othman et al., 2003 |
| $A_3$ | Gi | OPCs | N.A. | N.A. | Othman et al., 2003 |
| P2X1 | | OPCs | N.A. | N.A. | Agresti et al., 2005a |

| | | | | | |
|---|---|---|---|---|---|
| P2X2 | | OPCs | N.A. | N.A. | Matute et al., 2007; Agresti et al., 2005a |
| P2X3 | | OPCs | N.A. | N.A. | Agresti et al., 2005a |
| P2X4 | | OPCs | N.A. | N.A. | Matute et al., 2007; Agresti et al., 2005a |
| P2X7 | | All the stages | Sensor of axonal activity; contributes to the onset of disease | Upregulated before lesion formation in optic nerve; upregulated after MCAo | Matute et al., 2007; Agresti et al., 2005a |
| $P2Y_1$ | Gq | OPC | Chemotaxis | N.A. | Agresti et al., 2005a; Fumagalli et al., 2011 |
| $P2Y_2$ | Gq | OPCs | N.A. | Upregulated in "activated" OPCs | Moyon et al., 2015; Agresti et al., 2005a; Fumagalli et al., 2011 |
| $P2Y_4$ | Gq | OPCs | N.A. | N.A. | Agresti et al., 2005a |
| $P2Y_6$ | Gq | OPCs | N.A. | N.A. | Fields and Burnstock, 2006; Fumagalli et al., 2011 |
| $P2Y_{11}$ | Gq | OPCs | N.A. | N.A. | Fields and Burnstock, 2006 |
| $P2Y_{12}$ | Gi | OPCs and pre-oligodendrocytes (in vitro); myelinating stage (in vivo) | Promotes oligodendrocyte differentiation, adhesion of OPCs and expansion of processes in intermediate oligodendrocytes | Downregulated in close proximity of human MS lesions | Dennis et al., 2012; Amadio et al., 2010; Amadio et al., 2006; Fumagalli et al., 2011 |
| $P2Y_{13}$ | Gi | OPCs | N.A. | N.A. | Fields and Burnstock, 2006; Fumagalli et al., 2011 |
| $P2Y_{14}$ | Gi | OPC | N.A. | N.A. | Fumagalli et al., 2011 |
| GPR17 | Gi; outward K+ channels | OPCs and pre-oligodendrocytes | Promotes differentiation of early OPCs; impairment of its down-regulation inhibits terminal maturation | Upregulated after MCAo, in MS patients, EAE and focal demyelination | Ciana et al., 2006; Lecca et al., 2008; Pugliese et al., 2009; Chen et al., 2009; Boda et al., 2011; Fumagalli et al., 2011 |

**Figure 1. Upregulation of GPR17 in mouse models of CNS degeneration.**