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Sildenafil Inhibits Myelin Expression and Myelination of Oligodendroglial Precursor Cells

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Abstract

Phosphodiesterases (PDEs) have previously been implicated in oligodendrocyte maturation and myelination of central nervous system axons. Sildenafil citrate is a phosphodiesterase inhibitor known to block PDE5, which also reduces inflammation in the experimental autoimmune encephalomyelitis demyelinating model. To find out whether this inhibitor might exert beneficial effects on central nervous system myelin repair activities, we investigated to what degree sildenafil modulates differentiation and maturation of cultured primary rat oligodendroglial precursor cells (OPCs). To this end, gene and protein expression of 2′,3′-cyclic-nucleotide 3′-phosphodiesterase, myelin basic protein, and myelin oligodendrocyte glycoprotein, as well as of negative regulators of myelin expression (Hes1, Hes5, Id2, Id4, Rock2, and p57Kip2) were measured in OPCs treated with sildenafil. Moreover, the subcellular distribution of the p57kip2 protein was determined after sildenafil treatment, as this revealed to be an early predictor of the oligodendroglial differentiation capacity. *In vitro* myelination assays were done to measure the myelination capacity of oligodendrocytes treated with sildenafil. We found that sildenafil significantly diminished myelin gene expression and protein expression. Moreover, sildenafil also increased the expression of Id2 and Id4 negative transcriptional regulators, and the degree of OPCs with cytoplasmic p57kip2 protein localization was reduced, providing evidence that the PDE blocker impaired the differentiation capacity. Finally, sildenafil also interfered with the establishment of internodes as revealed by *in vitro* myelination assays. We therefore conclude that blocking PDE5 activities exerts a negative impact on intrinsic oligodendroglial differentiation processes.

Keywords

oligodendrocyte, myelin repair, remyelination, multiple sclerosis, viagra, neuroregeneration

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Introduction

Multiple sclerosis (MS) patients suffer from autoimmune attacks directed against central nervous system (CNS) cells, which results in the loss of oligodendrocytes (OLs) and myelin sheaths and subsequently impairs electrical signal conduction as well as axonal/neuronal survival. Although generally regarded as a regeneration incompetent organ, the adult CNS retains a limited capacity to restore myelin sheaths. Such endogenous repair activities are mainly mediated via activation, recruitment, and differentiation of resident oligodendroglial precursor cells (OPCs) eventually replacing lost OLs. Unfortunately, the overall remyelination remains

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inefficient as these cells often fail in successfully differentiating and in generating functional myelin sheaths, thus contributing to permanent neurological disability. Such restrictions are thought to result from the activity of intrinsic differentiation inhibitors (Kremer et al., 2011) as well as from the lesion environment (Kotter et al., 2011). Pharmacological modulation of oligodendroglial maturation responses is therefore of particular interest regarding the development of protective and repair therapies as they are currently missing for the treatment of MS patients. This study aimed at uncovering the effect of phosphodiesterase inhibitors (PIs) on oligodendroglial differentiation processes.

PIs are a group of molecules that inhibit cyclic phosphodiesterases (PDEs), thus regulating intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) levels (Azevedo et al., 2014) and revealed to act on many intracellular signaling pathways and to modulate various biological processes such as inflammation, gene expression, apoptosis, proliferation, and differentiation (Maurice et al., 2014). They act as hydrolases that convert cAMP and cGMP to 5′-AMP and 5′-GMP, respectively, with affinities for one or both cyclic nucleotides (Boswell-Smith et al., 2006; Azevedo et al., 2014; Maurice et al., 2014). Sildenafil citrate (SDN), a PDE5 inhibitor, is used as treatment for erectile dysfunction and pulmonary arterial hypertension (Dhariwal and Bavdekar, 2015) but was also demonstrated to inhibit inflammatory responses in humans (Raposo et al., 2013) and in the mouse model of experimental autoimmune encephalomyelitis (EAE; Pifarre et al., 2011). While decreasing CD3+ leukocyte infiltration and microglial/macrophage activation in the spinal cord and thereby decreasing EAE clinical symptoms (Pifarre et al., 2011), myelin basic protein (MBP) expression was augmented (Pifarré et al., 2014), suggesting a potential impact on CNS repair/protection. Moreover, in the cuprizone model of toxic demyelination (Torkildsen et al., 2008), widely used as a remyelination model, sildenafil application reduced myelin damage that was probably regulated via metalloproteinases (Nunes et al., 2012). As in one of our previous studies, we could show that other PDE inhibitors, such as vinpocetine (Torres et al., 2012) acting on PDE1, where inhibition was observed, have the capacity to modulate the expression of OPC differentiation inhibitors (Kremer et al., 2011); we now wanted to shed light on a potential impact of sildenafil on the myelination process.

Methods

OPC Culture

This work received approval from both, Science and Ethical Committees (number B17-13) from the National Institute of Respiratory Diseases, in México City. Results showed in this work are the product of the collaboration among National Institute of Respiratory Diseases, INNNMVS, and Heinrich-Heine-University. While differences among labs may occur, results were confirmed among labs. Newborn rats were euthanized using isoflurane inhalation. Primary OPCs were obtained from postnatal Day 0 cerebral rat cortices as previously described (Kremer et al., 2009; Göttle et al., 2010). OPCs were kept on Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (4 mM, Sigma-Aldrich, St. Louis, MO, USA), penicillin, streptomycin (200 U/ml, Sigma-Aldrich), and 10% bovine fetal serum during 2 weeks. Then, OPCs were purified as described (Kremer et al., 2009; Göttle et al., 2010) and plated in SATO medium (DMEM supplemented with bovine insulin [5 µg/ml, Sigma-Aldrich], human transferrin [50 µg/ml, Sigma-Aldrich], BSA V [100 µg/ml, Sigma-Aldrich], progesterone [6.2 ng/ml, Sigma-Aldrich], putrescine [16 µg/ml, Sigma-Aldrich], sodium selenite [5 ng/ml, Sigma-Aldrich], T3 [400 ng/ml, Sigma-Aldrich], T4 [400 ng/ml, Sigma-Aldrich], L-glutamine [4 mM, Sigma-Aldrich], penicillin, streptomycin [200 U/ml, Sigma-Aldrich]) and 0.5% bovine fetal serum and plated onto poly-D-lysine (PDL 0.5 mg/l, Sigma-Aldrich)-coated tissue culture plastics or PDL-coated coverslips for immunofluorescence. For spontaneous OPC differentiation to be initiated, cells were transferred into SATO medium supplemented with 0.5% fetal calf serum. Cells were then ready to be treated with PDE inhibitors or controls.

PI Treatment of OPCs

SDN (5-[2-Ethoxy-5-(4-methylpiperazin-1-yl) sulfonylphenyl]-1-methyl-3-propyl-4H-pyrazolo[5,4-e] pyrimidin-7-one citrate salt, Sigma-Aldrich) was diluted in ethanol and stored at –20°C. Other recommended solvents such as DMSO were observed to affect myelin expression (data not shown), and storage in distilled water led to low activity (data not shown). To establish PDE inhibitor concentrations compatible with OPC viability, trypan blue staining was performed in buffer-treated cells and cells treated with various sildenafil concentrations in Log10 scale (0.01 to 100 µM). A second dose curve was done with concentrations of 10, 20, 50, and 100 µM to establish the best dose where we have good viability without losing a possible effect. Conditions with more than 10% variation on viability were not considered, and at the end, a final concentration of 50 µM sildenafil was used for all stimulation experiments. Treatments were added to the medium on Day 0. Control cells were treated with identical ethanol dilutions. Differentiation medium was used in all time points and conditions. Medium was changed on Days 3

and 7 with replenishment of the treatments. All experiments were done in triplicates. Cells were plated duplicated PDL-coated wells for quantitative polymerase chain reaction techniques or at least duplicated wells containing PLD-coated coverslips for immunofluorescence techniques.

Detection of Myelin- and p57Kip2 Protein Expression

Markers of early (2′,3′-cyclic-nucleotide 3′-phosphodiesterase [CNPase] measured after 24 hr of stimulation) and late (MBP measured after 72 hr of stimulation) OPC differentiation markers, as well as of proteins related to OPC's capacity to differentiate (p57Kip2; Göttle et al., 2015) were determined by immunofluorescent staining on 4% paraformaldehyde-fixed cultures as described previously (Kremer et al., 2009). Coverslips with fixed cells were recovered from wells for staining. Primary antibodies were diluted, using a final volume of 100 µl of antibody dilution per coverslip as follows: mouse anti-CNPase antibody (1:500, Millipore, Billerica, MA), anti-MBP antibody (1:500, Millipore), anti-p57kip2 antibody (1:500, Sigma-Aldrich, Taufkirchen, Germany), rabbit anti-cleaved caspase 3 antibody (1:500, Cell Signaling, Frankfurt am Main, Germany), and rabbit anti-Ki67 (1:500, Abcam, Cambridge, UK); anti-IgG secondary antibodies conjugated with Alexa Fluor 488 and Alexa Fluor 594 (1:500, Life Technologies, NY, USA) were used for signal visualization. Nuclei were stained with 4′,6-diamidino-2-phenylindol (Roche, Basel, Switzerland). Coverslips were mounted on glass slides for microscopy. Marker expression was analyzed by fluorescence microscopy (Zeiss ZEN Digital Imaging, Carl Zeiss, Jena, Germany). ImageJ software (NIH) was used to automatically count of nucleus. Percentage of positive cells expressing a marker was calculated in relation to the total number of nuclei per picture. Image analysis was performed in 9 pictures per coverslip for a total 54 pictures per condition from 3 independent experiments by duplicates coverslips, with an average (± SD) of 111.09 ± 61.33 cells per picture. Prism5 GraphPad Software was used for statistical analysis. Results are plotted with all data from triplicated experiments as bars ± standard error of mean (SEM), except for Figure 2, were SD was used.

Gene Expression Analysis

Total RNA purification was done using the RNeasy procedure (Qiagen, Hilden, Germany). RNA was reverse transcribed using the high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative determination of gene expression levels was performed on StepOne detection system (Applied Biosystems) using SybrGreen Universal Master Mix (Applied Biosystems). To determine the transcript levels of CNPase, MBP, myelin oligodendrocyte glycoprotein, p57kip2, Hes1, Rock2, Id2, and Id4 genes, previously described sequences were used (Torres et al., 2012). Additional primers for the detection of Hes5 transcript levels were generated: GGTTCCAGA GGCCAAACATC, GTTGCCACATTGACCGTGAC. Glyceraldehyde-3-phosphate dehydrogenase was used as reference gene, and relative gene expression levels were determined according to the ΔΔCt method (Applied Biosystems). Triplicates of independent experiments were performed with duplicated wells per condition. Each sample was quantitated by duplicate. Results are shown with data from the triplicates in the Figures (mean values ± Tukey's range). Prism5 GraphPad Software was used for statistical analysis. Two-way analysis of variance test was done to determine statistical significance in time-course experiments and post hoc multiple *t* tests were done to determine difference between controls and treatment at a specific time point.

Myelinating Coculture

Dissociated neuron/oligodendrocyte cocultures were obtained from embryonic Day 16 (E16) rat cerebral cortex (Wistar rats of either sex) according to Pang et al. (2012) and as previously published by us (Göttle et al., 2015). Cortical cells were plated on 15-mm poly-D-lysine (0.1 mg/ml)-coated cover slips (65,000 cells per cover slip) and kept in myelination medium consisting of N2 and neurobasal medium (ThermoFisher Scientific, Darmstadt, Germany; ratio 1:1) including nerve growth factor (NGF) (50 ng/ml) and neurotrophin 3 (NT-3) (10 ng/ml; both R&D Systems, Minneapolis, USA). The day of primary culture was defined as Day 1 *in vitro*. After 10 days *in vitro*, insulin was excluded and the ratio of the insulin-free N2 to neurobasal medium including B27 supplement (ThermoFisher Scientific) was adjusted to 4:1. This myelination medium was further supplemented with 60 ng/ml tri-iodo-thyronine (T3, Sigma-Aldrich, Taufkirchen, Germany). Final concentrations of individual N2 medium components (DMEM-F12 based, high glucose, ThermoFisher Scientific) were insulin (10 µg/ml), transferrin (50 µg/ml), sodium selenite (5.2 ng/ml), hydrocortisone (18 ng/ml), putrescine (16 µg/ml), progesterone (6.3 ng/ml), biotin (10 ng/ml), N-acetyl-L-cysteine (5 µg/ml; all Sigma-Aldrich, Taufkirchen, Germany), bovine serum albumin (0.1%, Roth, Karlsruhe, Germany), and penicillin–streptomycin (50 units/ml, ThermoFisher Scientific). At 30 days *in vitro* (DIV30), cover slips were washed with PBS, fixed with 4% paraformaldehyde, and processed for immunofluorescent staining. At the onset of myelination 17 days *in vitro*, cultures were treated for another 13 days with myelination medium supplemented with 50 µM sildenafil

until DIV30. Myelination experiments were performed three times, and data are shown as mean values ± SEM.

Results

Sildenafil Inhibits Myelin Gene Expression

In the EAE animal model, clinical symptoms can be diminished by application of PIs (Pifarré et al., 2014; Mestre et al., 2015), and these inhibitors addressing PDE5 and PDE7 have also been tested for MS treatment (Goodman et al., 2016). However, it is currently uncertain for many PDEs and their corresponding PIs whether they also exert direct oligodendroglial effects and whether they can for example modulate myelin synthesis. We investigated the effect of PIs on myelin transcript levels. To this end, cultured primary rat OPCs were treated with sildenafil (inhibitor of PDE5 specific of cGMP) for 1, 3, 7, and 10 days. RNA was extracted, and gene expression was determined by real-time quantitative reverse transcription polymerase chain reaction. Sildenafil inhibited CNPase, MBP, and myelin oligodendrocyte glycoprotein transcript levels over the course of several days in culture but with different (gene specific) extents and dynamics (Figure 1).

Sildenafil Inhibit Myelin Protein Expression

Given the more pronounced effect of sildenafil, we focused our study on this particular PDE inhibitor. To determine the effect on myelin protein expression, OPCs were nonstimulated (ctrl) or treated with sildenafil in SATO medium, and then subjected to immunofluorescence analysis to determine to what degree the cell's capacity to express myelin proteins was affected. We observed that sildenafil significantly reduced the number of OPCs expressing either CNPase (after 24 hr) or MBP (after 72 hr; Figure 2).

Sildenafil Alter Gene Expression of Negative Regulators

Oligodendroglial differentiation inhibitors modulate myelin expression and differentiation in OLs (Kremer et al., 2011; Göttle et al., 2015), acting on different signaling pathways. In a previous study, we found that yet another PI, vinpocetine, downregulates the differentiation of OPCs and at the same time exerts an impact on the expression of some oligodendroglial differentiation inhibitors (Torres et al., 2012). We therefore tested whether sildenafil also changed transcript levels of such negative regulators. Importantly, upregulation of

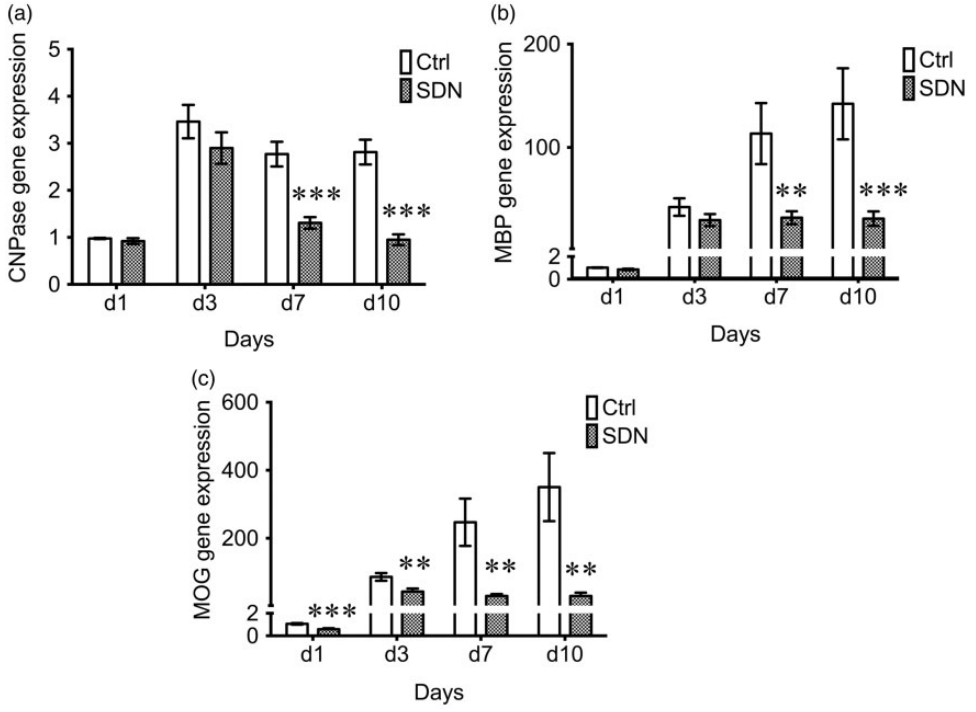

Figure 1. Temporal expression analysis of myelin genes. Determination of transcript levels by real-time quantitative RT-PCR of oligodendroglial cells after treatment with sildenafil (SDN, 50 μM) demonstrated that myelin genes CNPase (a), MBP (b), and MOG (c) were significantly downregulated at some time points when compared with control cells. GAPDH gene expression was used as reference. Data are mean values from three independent experiments ± SEM (multiple *t* tests were performed, $*p < .05$; $**p < .01$; $***p < .001$).
CNPase = 2′,3′-cyclic-nucleotide 3′-phosphodiesterase; MOG = myelin oligodendrocyte glycoprotein; SDN = sildenafil citrate; MBP = myelin basic protein.

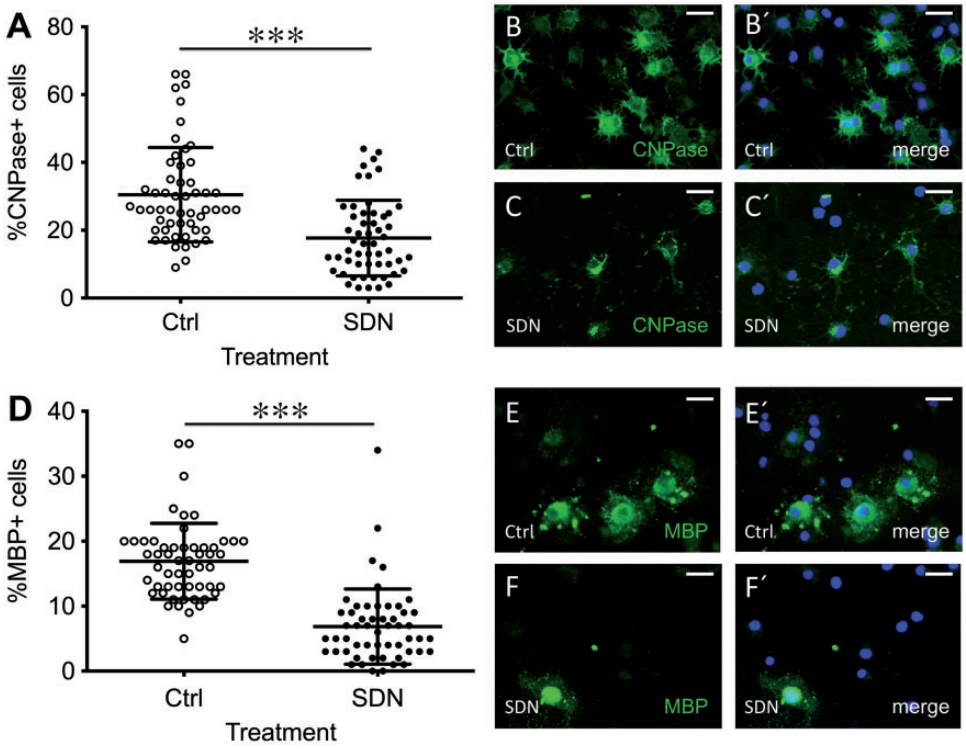

Figure 2. Effect of sildenafil (SDN) phosphodiesterase inhibitor on myelin protein expression. The degrees of myelin-positivity between untreated (ctrl) or sildenafil (SDN, 50 μM) treated OPCs were determined after 24 hr (CNPase) and 72 hr (MBP). (a, d) Quantitative evaluation of CNPase-positive- and MBP-positive OPCs. (b–c', e–f') Representative pictures of immunostained cells (CNPase after 24 hr, MBP after 72 hr) following buffer (ctrl) or sildenafil (SDN, 50 μM) treatment. Nuclei were stained with DAPI (blue). Data are mean values from three independent experiments ± SD (unpaired *t* test, *** $p < .0001$). Magnification bars: 25 μm. CNPase = 2′,3′-cyclic-nucleotide 3′-phosphodiesterase; SDN = sildenafil citrate; MBP = myelin basic protein.

differentiation inhibitors, which could account for the differentiation blockade, was observed on Day 7 and Day 10 for Id2 and Id4 genes upon sildenafil treatment. Sildenafil show no difference on Rock2, Hes1, or Hes5 gene expression (Figure 3).

p57Kip2 Localization Is Altered by Sildenafil

Because the differentiation competence of OPCs was shown to be dependent on nuclear exclusion of p57kip2 (Göttle et al., 2015) and because we also observed that not all of the previously described oligodendroglial differentiation inhibitors responded to sildenafil exposure, we were interested to see whether this treatment affects the subcellular distribution of the p57kip2 protein (Figure 4). We therefore scored for either nuclear (clear bars) or cytoplasmic (open bars) p57kip2 protein localization for the time points 1 and 3 days (Figure 4(a) and (d)). In comparison with control-stimulated cells, where the p57kip2 protein was successfully expelled from the nucleus (cytoplasmic: 14.9% at Day 1 and 29.4% at Day 3) over time and during natural maturation, sildenafil treated cells featured primarily nuclear p57kip2 proteins (cytoplasmic: 1.3% at Day 1 and 2.4% at Day 3; Figure 4(b'), (c'), (e'), (f')). Sildenafil is therefore likely to act already at this early differentiation competence step leading to severely diminished levels of myelin protein expression as also revealed in these experiments with a 5.4-fold lowered degree of CNPase-positive- and a 4.6-fold lowered degree of MBP-positive oligodendroglial cells (data not shown) and representative pictures (Figure 4(b'''), (c'''), (e'''), (f''')).

Sildenafil Dependent Myelination Deficits

Finally, it was important to extend our findings onto the myelination process (Figure 5(a) to (c)). To this end, mixed rat neuron/glia cocultures were grown for 17 days and then treated for another 13 days with myelination medium with or without addition of sildenafil. After a total duration of 30 days in culture, the percentage of MBP-positive OLs exhibiting a myelinating phenotype (i.e., featuring internodes) was determined based on the total number of Olig2-positive cells. This analysis revealed a strong decrease in the degree of myelinating OLs in presence of sildenafil indicating that blocking of PDE5 is preventing this important neuron/glia interaction. Additional analyses confirmed that the number of

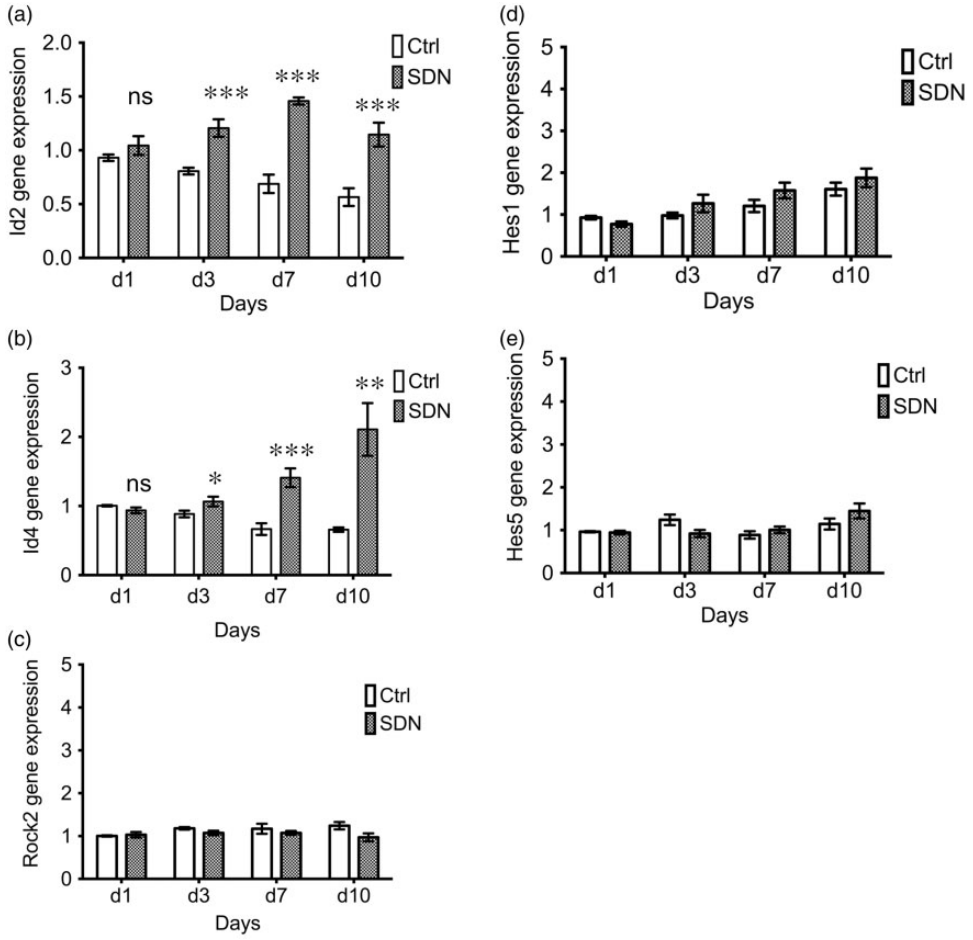

Figure 3. Temporal expression analysis of genes encoding oligodendroglial differentiation inhibitors in response to sildenafil treatment. Determination of transcript levels by quantitative real-time PCR after treatment of oligodendroglial cells with sildenafil citrate (SDN, 50 μM) demonstrated that expression of negative regulators genes Id2 (a) and Id4 (b) were significantly and consistently upregulated, while Rock2 (c), Hes1 (d), and Hes5 (e) remained unchanged. GAPDH gene expression was used as reference. Data are shown as mean values from three independent experiments ± SEM (multiple *t* tests, control vs. treatment, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$).
SDN = sildenafil citrate.

Olig2-positive OPCs was not affected by the treatment and that no changes in apoptotic cell death (cleaved caspase 3-positivity) or proliferation (Ki67 positivity) occurred (Figure 5(a') to (a''')).

Discussion

Phosphodiesterase inhibitors, such as vinpocetine, cilostazol, rolipram, VP3.15, sildenafil, and ibudilast, are widely used as anti-inflammatory agents and constitute potential candidates to treat autoimmune demyelination disorders because they were shown to ameliorate the clinical signs and to protect white matter in EAE. Along these lines, prevention of axonal loss, promotion of remyelination, and attenuation of inflammation were observed (Paintlia et al., 2008; Kureshiro et al., 2009; Barkhof et al., 2010; Medina, 2010; Pifarre et al., 2011; Martín-Álvarez et al., 2017). On the other hand, rolipram appears to have a positive effect on myelin expression in cell cultures, affecting Erk1/2 or p38Mapk-Creb1 activities (Syed et al., 2013). Also, PDE7 inhibitors have previously shown to promote OPC differentiation and survival (Medina-Rodríguez et al., 2013).

Our results showed that myelin protein and gene expression is inhibited by sildenafil. It is generally known that in myelinating (precursor) cells, both myelin gene as well as myelin protein expression are regulated, in that, for example, transcripts can accumulate, but only few proteins are produced unless axonal contacts are established. Our observations point to a broad suppressing role of sildenafil in that it can act on myelin gene transcription but also in exerting a negative impact on myelin protein production. To what degree this is directly linked or whether additional independent mechanisms apply was not determined and is currently out of scope of this article. However, it is clear from our data

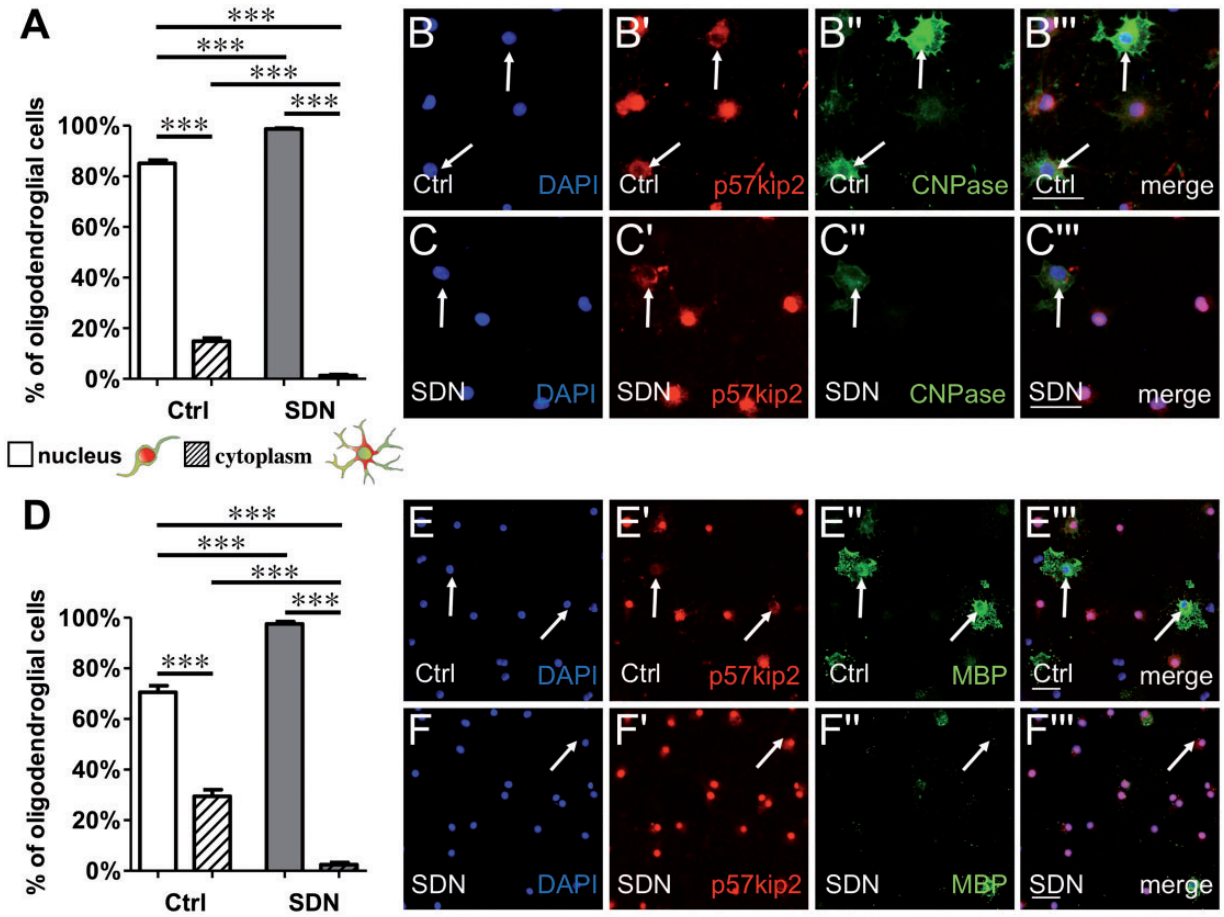

Figure 4. Effect of sildenafil on nucleocytoplasmic p57kip2 protein translocation. (a) Percentage of OPCs after 1 day in medium only (control) or in medium supplemented with sildenafil (SDN) with nuclear- (open bars) and cytoplasmic localization (dashed bars) of the p57kip2 protein. (b–c''') Representative pictures of CNPase- and p57kip2- immunostained OPCs treated for 24 hr with sildenafil (SDN, 50 μM) or buffer (ctrl). (d) Percentage of OPCs after 3 days in medium only (control) or in medium supplemented with sildenafil (SDN) with nuclear- (open bars) and cytoplasmic localization (dashed bars) of the p57kip2 protein. (e–f''') Representative pictures of MBP- and p57kip2-immunostained cells treated for 72 hr with sildenafil (SDN, 50 μM) or buffer (ctrl). One representative out of three independent measurements is shown; data are mean values ± SEM (Student's *t* test of treatment vs. control: $*p < .05$). Magnification bar: 25 μm. SDN = sildenafil citrate; CNPase = 2′,3′-cyclic-nucleotide 3′-phosphodiesterase; MBP = myelin basic protein; DAPI = 4′,6-diamidino-2-phenylindol.

that SDN downregulates myelin transcription, myelin protein production as well as the establishment of myelin sheaths. Overall, this constitutes a strong negative impact.

The reduction of oligodendroglial differentiation as well as the myelination process by sildenafil is most likely mediated via a prevented translocation of the p57kip2 inhibitory protein from nucleus to cytoplasm. We have previously shown that the subcellular localization of the p57kip2 protein constitutes a functional differentiation rate limiting process (Göttle et al., 2015). And in two follow-up publications from our laboratory (Göttle et al., 2018, 2019), the correlation between nuclear and cytoplasmic localization of this protein with negative (pHERV-W ENV) and positive (teriflunomide) regulators of OPC differentiation and myelination could be established. For SDN again, a correlation with retained nuclear presence and impeded OPC differentiation is shown, indicating that at least p57kip2 is also involved in this process.

Other phosphodiesterase inhibitors have also been suggested for myelin repair. Cilostazol, a PDE3 inhibitor, that has been used for the treatment of brain diseases, such as Alzheimer's disease and stroke (Schaler and Myeku, 2018), revealed to diminish inflammation symptoms in EAE models (Kureshiro et al., 2009). Rolipram (a PDE4 inhibitor) was shown to boost OPC differentiation and maturation both *in vitro* as well as *in vivo* (Syed et al., 2013).

In a previous study (Torres et al., 2012), we evaluated the *in vitro* effect of vinpocetine, a widely used PDE1 inhibitor, showing that it not only decreased overall

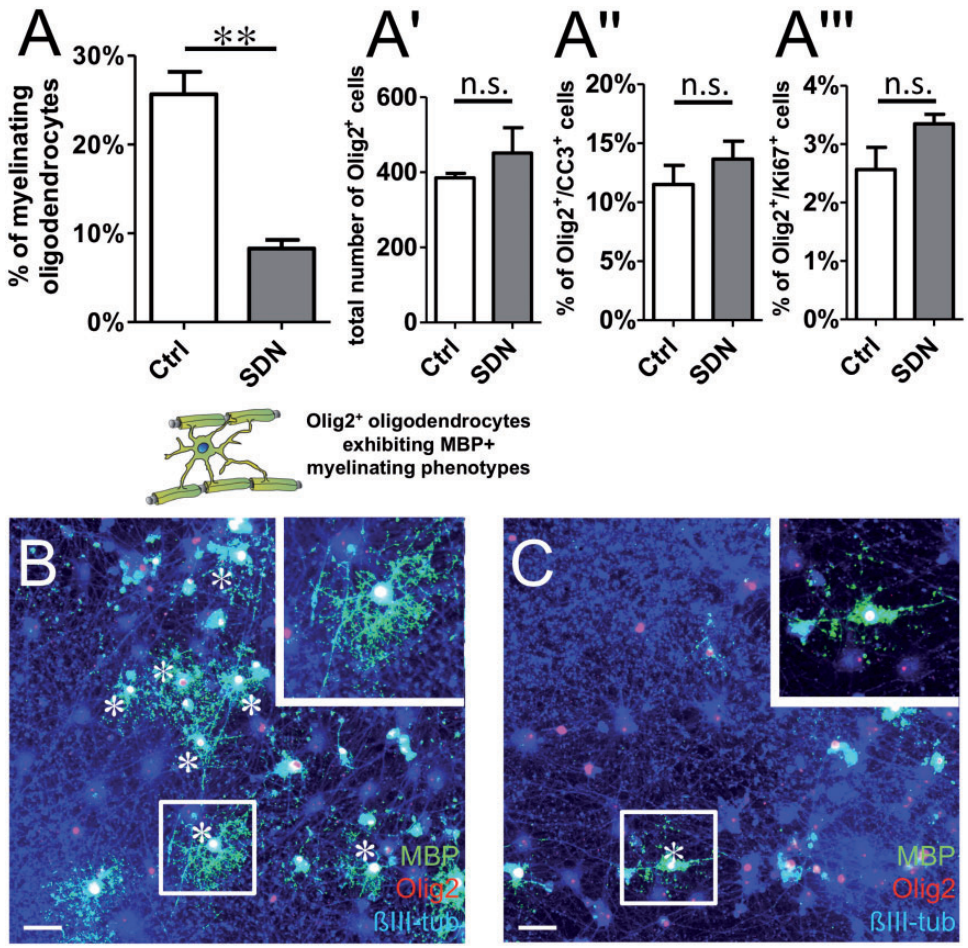

Figure 5. Breakdown of myelination *in vitro* upon sildenafil exposure. (a) Percentage of myelinating Olig2- and MBP-positive oligodendroglial cells generating internodes in untreated or in sildenafil (50 μM) treated neuron/glia cocultures at DIV30. (a') Quantification of the total number of Olig2-positive cells (red). (a'') Percentage of double Olig2/CC3+ positive cells. (a''') Percentage of double Olig2/Ki67+ positive cells. (b, c) Representative pictures of *in vitro* myelinating cocultures displaying a decrease in the number of (MBP in green) myelinated segments (asterisks) in presence of sildenafil (axons are visualized by means of βIII-tubulin staining in blue). Data are shown as mean values; error bars represent SEM. Number of experiments: $n = 3$ for (a); *t* test (**$p < .01$). Inserts in top right corners correspond to enlarged fields marked by the white squares in (b) and (c). Magnification bar: 50 μm. SDN = sildenafil citrate; MBP = myelin basic protein.

myelin synthesis but also boosted the expression of negative regulatory genes such as Id2, Id4, and Hes1 and furthermore modulated expression levels of differentiation-associated factors Rock2 and p57Kip2. Apart from the negative cell differentiation effect by sildenafil, substantial differences were found in regard to modulation of oligodendroglial inhibitors. This indicated that different intrinsic signaling pathways are affected by vinpocetine and sildenafil, which might then also account for observed differences in myelin gene expression dynamics. Nevertheless, the degree of myelin protein expressing cells was significantly reduced by both inhibitors.

Of note, a recent article described a promoting effect of sildenafil on remyelination (Díaz-Lucena et al., 2018). However, it was suggested that the observed effect is rather related to the modulation of immune cells and not to a direct effect on oligodendroglial cells. In their model, the authors used lipopolysaccharides to stimulate astrocytes and microglia for chemokine production, which, in turn, promoted myelin synthesis by neighboring oligodendroglial cells. Of note, we described direct PI-mediated effects on OPCs in absence of proinflammatory or demyelinating stimuli and without participation from astrocytes or microglia. To interpret the currently available findings on the different PIs in the context of myelin repair, it becomes evident that indirect effects and adjacent cell populations, which can influence the myelin repair process, must be considered as well.

The current studies thus indicate that inhibition of PDE1 and PDE5 can be attributed to suppression of inflammatory responses, which in turn would allow parallel myelin regeneration to proceed. On the other side, it must be taken into account that inhibition of PDE5 by

sildenafil, revealed to interfere substantially with oligodendrogenesis. Therefore, an ideal anti-PDE directed drug should be able to reduce inflammation and at the same time either neglect or positively affect oligodendroglial cells to qualify as a promising treatment for white matter lesion repair.

Declaration of Conflicting Interests

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