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The Diadenosine Homodinucleotide P18 Improves In Vitro Myelination in Experimental Charcot-Marie-Tooth Type 1A

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ABSTRACT

Charcot-Marie-Tooth 1A (CMT1A) is a demyelinating hereditary neuropathy whose pathogenetic mechanisms are still poorly defined and an etiologic treatment is not yet available. An abnormally high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) occurs in Schwann cells from CMT1A rats (CMT1A SC) and is caused by overexpression of the purinoceptor P2X7. Normalization of the Ca^{2+} levels through down-regulation of P2X7 appears to restore the normal phenotype of CMT1A SC in vitro. We recently demonstrated that the diadenosine 5′,5‴-P1, P2-diphosphate (Ap2A) isomer P18 behaves as an antagonist of the P2X7 purinergic receptor, effectively blocking channel opening induced by ATP. In addition, P18 behaves as a P2Y11 agonist, inducing cAMP overproduction in P2Y11-overexpressing cells. Here we investigated the in vitro effects of P18 on CMT1A SC. We observed that basal levels of intracellular cAMP ($[cAMP]_i$), a known regulator of SC differentiation and myelination, are significantly lower in CMT1A SC than in wild-type (wt) cells. P18 increased $[cAMP]_i$ in both CMT1A and wt SC, and this effects was blunted by NF157, a specific P2Y11 antagonist. Prolonged treatment of organotypic dorsal root ganglia (DRG) cultures with P18 significantly increased expression of myelin protein zero, a marker of myelin production, in both CMT1A and wt cultures. Interestingly, P18 decreased the content of non-phosphorylated neurofilaments, a marker of axonal damage, only in CMT1A DRG cultures. These results suggest that P2X7 antagonists, in combination with $[cAMP]_i$-increasing agents, could represent a therapeutic strategy aimed at correcting the molecular derangements causing the CMT1A phenotype. J. Cell. Biochem. 115: 161–167, 2014.

KEY WORDS: cAMP; CMT1A; DRG CULTURES; MYELINATION; P2X7

ADP-ribosyl cyclases (ADPRC) are a family of multifunctional enzymes that metabolize NAD^+ and produce several molecules involved in intracellular calcium signaling and in the regulation of Ca^{2+}-dependent cell functions [Lee, 2001; Koch-Nolte et al., 2009]. NAD^+ is converted by ADPRCs to cyclic ADP-ribose (cADPR), mobilizing Ca^{2+} from intracellular stores [Lee, 2001; Guse, 2005] and to ADP-ribose, which opens TRPM2 channels with extracellular Ca^{2+} influx [Perraud et al., 2001; Sano et al., 2001]. Different ADPRCs, including CD38 (the main ADPRC expressed in human cells), synthesize three diadenosine homodinucleotides from cADPR and adenine: diadenosine 5′,5‴-P1, P2-diphosphate (Ap2A) and two isomers thereof (P18 and P24), characterized by an unusual N-glycosidic bond between the incoming adenine and ribose [Basile et al., 2005]. Ap2A and its isomers, despite their structural similarity, exert different effects on the purinergic receptors P2X7 and P2Y11. P2X7 is a ligand-gated ion channel, characterized by two states of permeability: at high micromolar concentrations of ATP, P2X7 behaves as a cation-selective channel. Prolonged exposure to ATP triggers the transition to a non-selective pore, permeable to molecules of mass up to 900 Da [Di Virgilio, 1995]. P2Y11 is a G protein-coupled purinergic receptor: it is the only purinergic receptor known to stimulate adenylyl cyclase (AC), with subsequent

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intracellular cAMP production. In addition, P2Y11 is the only purinergic receptor coupled to both AC and phospholipase C [Qi et al., 2001]. We demonstrated that P18 behaves as an antagonist of P2X7 [Bruzzone et al., 2010]. Unlike P18, P24 is a partial agonist of P2X7, eliciting influx of extracellular Ca^{2+}, without leading to pore formation [Bruzzone et al., 2010]. Finally, Ap2A is neither an agonist nor an antagonist of P2X7 [Bruzzone et al., 2010]. Conversely, all three dinucleotides behave as P2Y11 agonists, inducing cAMP overproduction in P2Y11-overexpressing cells [Magnone et al., 2008].

Charcot-Marie-Tooth 1A (CMT1A), a demyelinating hereditary neuropathy, is associated with a duplication of chromosome 17p11.2, containing the gene encoding peripheral myelin protein 22 (PMP22) [Matsunami et al., 1992]. Results obtained in experimental CMT1A demonstrate that the increased *PMP22* gene dosage is responsible for the disease [Nobbio et al., 2001; Sereda et al., 2003; Fledrich et al., 2012; Li et al., 2013]. However, the limited knowledge of the molecular mechanisms underlying CMT1A has prevented the development of pathogenetic treatments. Based on data obtained on a mouse model [Passage et al., 2004], ascorbic acid (AA) was tested on CMT1A patients, with negative results [Pareyson et al., 2011].

Schwann cells (SC) regulate neuronal properties by synthesizing and organizing the peripheral myelin sheath [Pereira et al., 2012]. Primary SC cultures from CMT1A rats (CMT1A SC) develop an abnormal phenotype, characterized by defective differentiation and myelination [Nobbio et al., 2004]. Moreover, these cells show an abnormally high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), due to *PMP22*-dependent P2X7 overexpression. Decrease of the $[Ca^{2+}]_i$ with P2X7 antagonists or through P2X7 silencing restores the normal phenotype in CMT1A SC, this establishing a pathogenetic link between P2X7-mediated high $[Ca^{2+}]_i$ and SC impairment [Nobbio et al., 2009]. Treatment of CMT1A SC with P18 reverts the high $[Ca^{2+}]_i$ of these cells to normal values [Bruzzone et al., 2010].

As CMT1A SC show impaired differentiation [Nobbio et al., 2004] and cAMP is known to positively regulate SC differentiation and myelination [Jessen et al., 1991; Monk et al., 2009; Monje et al., 2010; Guo et al., 2012], we compared the $[cAMP]_i$ in wild-type (wt) and CMT1A SC. The lower $[cAMP]_i$ levels in CMT1A SC compared to wt cells prompted us to investigate whether P18 was able to increase the $[cAMP]_i$ in SC, as in other cell types [Magnone et al., 2008], and to establish the effect of chronic treatment with P18 on the phenotype of organotypic dorsal root ganglia (DRG) cultures from CMT1A rats. P18 improved myelination in both wt and CMT1A DRG cultures, as inferred from myelin protein zero (MPZ) and myelin basic protein (MBP) expression. Interestingly, P18 also reduced non-phosphorylated neurofilament (NF) content of CMT1A cultures, suggesting a beneficial action of the dinucleotide on the axon.

MATERIALS AND METHODS

MATERIALS

P18 and P24 were produced and purified as in Basile et al. [2005]. Ap2A was synthesized from AMP and HPLC-purified as described [Millo et al., 2008]. All other chemicals were obtained from Sigma–Aldrich (Milano, Italy).

ANTIBODIES

Monoclonal antibodies against MPZ (P07 extracellular domain, Astexx Ltd. & Co. KEG, Graz, Austria), myelin basic protein (MBP, MAB 386, Millipore, Milano, Italy), NF H non-phosphorylated (SMI-32) (Sternberger Monoclonals, Inc., Baltimore, MD), total NF (N52, Sigma–Aldrich) and α-actin (A3853, Sigma–Aldrich) were used. Secondary antibody reactions were carried out using antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA).

ANIMAL MODEL

Transgenic Sprague-Dawley rats overexpressing *PMP22* (CMT1A rats) were genotyped by PCR [Sereda et al., 1996]. Heterozygous animals and wt age-matched littermates were used. Animal rearing conditions were consistent with the guidelines of the Italian Ministry of Health and the study was approved by the San Martino Hospital Ethical Committee (Genova, Italy).

Primary cultures of SC. Primary SC cultures were isolated from newborn and adult rat sciatic nerves, as previously described [Brockes et al., 1979; Nobbio et al., 2004]. SC were grown in DMEM/F12 medium (Life Technologies) supplemented with 10% fetal calf serum, penicillin, and streptomycin for 48 h. Cytosine arabinoside (10^{-5} M) was added after 48 h and the treatment prolonged for further 48 h resulting in SC cultures that were 99% pure.

Organotypic DRG cultures. Myelinating DRG cultures were established from wt and CMT1A 15-day-old rat embryos, as previously described [Nobbio et al., 2001]. Embryos were genotyped from the remaining tissue, and the code was broken after quantitative evaluation of each culture. After sterile recovery of the embryos, 35–40 DRG were removed from each one, pretreated with trypsin (Hank's solution 0.25), and minced to provide a suspension of DRG cells in neurobasal medium (Life Technologies), supplemented with 15% newborn calf serum, AA (100 μg/ml final dilution) and nerve growth factor (5 ng/ml final dilution). This suspension was plated on a collagen substrate in flexible molded plastic ACLAR dishes, and 1×10^4 cells were placed in each dish. DRG cultures were grown for 30 days in the presence of P18 or Ap2A (or the vehicle).

DETERMINATION OF $[cAMP]_i$

Wt and CMT1A SC were cultured in 60 mm × 10 mm dishes: the medium was removed and cells were rinsed twice with 1 ml Hank's balanced salt solution (HBSS). Cells were treated (or not) with 10 μM P18 or P24 or Ap2A for 15 min at 37°C. At the end of the incubation, cells were then scraped in 300 μl water solution: 20 μl perchloric acid (9 M) was added to 250 μl of the cell extract, while the rest of the cell lysate was used for protein content determination [Bradford, 1976]. Alternatively, wt and CMT1A SC were exposed to 0.2 μM P18 or P24 or Ap2A, adding new media containing (or not) the different compounds every other day. After 5 days, cells were rinsed with HBSS and acidic cell extracts were prepared as above. Cell extracts were neutralized as previously described [Moreschi et al., 2006] and $[cAMP]_i$ was measured with a specific ELISA kit (Cayman, Ann Arbor, MI).

WESTERN BLOT EXPERIMENTS

Wt and CMT1A myelinating DRG cultures were treated for 30 days with 0.2 μM P18 or Ap2A (or the vehicle), adding fresh medium containing the different compounds every other day. To analyze MPZ expression levels, DRG lysates were prepared in 100 μl lysis buffer (95 mM NaCl, 25 mM Tris–HCl, pH 7.4, 10 mM EDTA, 2% SDS, 0.01% protease inhibitor mixture). Total protein content was determined by the Bradford assay (Bio-Rad, Hercules, CA), and equal amounts of proteins (25 μg) were loaded onto a 12% polyacrylamide gel and separated by SDS–PAGE. Proteins were transferred to nitrocellulose membrane (Bio-Rad) and incubated with the specific primary antibody against MPZ overnight and with the antibody against α-actin for 1 h. Following incubation with the appropriate secondary antibodies and ECL detection (GE Healthcare, Milano, Italy), band intensity was measured with the ChemiDoc imaging system (Bio-Rad) and the amount of MPZ was expressed relative to α-actin. For the evaluation of non-phosphorylated NF, as a marker of axonal pathologic changes [Trapp et al., 1998; Nobbio et al., 2006], total lysates of DRG cultures from wt and CMT1A rats were prepared directly in 70 μl of BUST buffer (0.5% SDS, 8 M urea, 2% β-ME, 0.01% protease inhibitor cocktail, 0.l M Tris–HCl, pH 6.8). Total protein content was determined by the Bio-Rad Protein Detection kit and equal protein amounts from normal and transgenic animals were loaded on a 7.5% polyacrylamide gel and separated by SDS–PAGE. Proteins were transferred to 0.2 μm pore sized nitrocellulose membranes (Amersham, Arlington Heights, IL), which were incubated with a primary antibody towards non-phosphorylated NF (SMI32) overnight, or with an antibody towards total NF (N52) for 1 h. Following incubation with the appropriate secondary antibodies and ECL detection (GE Healthcare), band intensity was measured with the ChemiDoc imaging system (Bio-Rad) and the amount of non-phosphorylated NF was expressed relative to total NF.

IMMUNOCYTOCHEMISTRY

Wt and CMT1A myelinating DRG cultures were treated for 30 days with 0.2 μM P18 (or the vehicle), adding fresh medium containing the different compounds every other day. DRG cultures were then fixed for 30 min in 4% paraformaldehyde in PBS, permeabilized for 5 min in cold methanol at −20°C and incubated for 1 h in blocking buffer (10% normal goat serum in PBS). Incubation with the primary antibodies was performed for 18 h at 4°C with a monoclonal antibody against MBP (1:100) and a polyclonal antibody against NFs (1:100) in blocking buffer. After washing, cells were incubated with the secondary goat anti-mouse ALEXA594-IgG and goat anti-rabbit ALEXA488-IgG antibodies at a 1:250 dilution for 1 h at 25°C. Cell nuclei were stained with DAPI and ACLAR dishes were sealed on slides. Images were taken with an Olympus PROVIS AX60 microscope, connected to an Olympus DP70 digital camera.

STATISTICAL ANALYSIS

All data are expressed as mean ± SD. Differences between two groups were determined using unpaired Student's *t*-test. Multiple-parameter comparisons were performed by one-way ANOVA followed by Bonferroni's post hoc test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

BASAL cAMP LEVELS ARE LOWER IN RAT CMT1A SC THAN IN WT SC

Since cAMP plays an established role in regulating SC differentiation and myelination [Jessen et al., 1991; Monk et al., 2009; Monje et al., 2010; Guo et al., 2012], we compared the basal $[cAMP]_i$ in wt and CMT1A SC. The $[cAMP]_i$ was significantly lower (by approximately 30%) in CMT1A SC than in the wt ones, isolated from sciatic nerves of 3- as well as of 30-day-old rats (Fig. 1A).

cAMP LEVELS ARE INCREASED BY P18 IN SC

Presence of P2Y11 in rat cells is controversial [von Kügelgen, 2006], although its expression has been demonstrated by Western blot and immunofluorescence staining and by its ATP-induced functional activation with consequent cAMP production in rat neutrophils [Alkayed et al., 2012], bile duct epithelia [Yu et al., 2009], and glial cells [Brandenburg et al., 2010].

A 15 min incubation with 10 μM P18, which behaves as an agonist of human P2Y11 [Magnone et al., 2008], resulted in a significant $[cAMP]_i$ rise (by approximately 1.6-fold), in both wt and CMT1A SC (Fig. 1B). Both P18 isomers, Ap2A and P24, act as agonists on the human P2Y11 purinoceptor, similarly to P18 [Magnone et al., 2008]. When SC were incubated in the presence of 10 μM Ap2A for 15 min, $[cAMP]_i$ was increased by 1.5- and 1.4-fold in wt and CMT1A SC, respectively. A similar incubation with P24 resulted in a 4.3- and 3.9-fold increase of the $[cAMP]_i$ in wt and CMT1A SC, respectively (not shown). The P18-induced $[cAMP]_i$ increase was completely abolished in the presence of NF157 (Fig. 1B), a specific P2Y11 receptor antagonist [Ullmann et al., 2005]. This indicates that the P18-induced $[cAMP]_i$ increase is likely mediated by P2Y11 itself or by a P2Y11-like receptor in SC. Moreover, a 5-day incubation with 0.2 μM P18 was sufficient to significantly increase $[cAMP]_i$ in wt and in CMT1A SC (Fig. 1C).

P18 IMPROVES THE PATHOLOGICAL PHENOTYPE OF CMT1A DRG CULTURES

Thus, P18 appears to possess a double-faced action: on one side, it lowers the $[Ca^{2+}]_i$ to normal values in CMT1A SC [Bruzzone et al., 2010], and, on the other hand, it increases the $[cAMP]_i$ in the same cells. We next investigated whether treatment with P18 could ameliorate myelination in organotypic DRG cultures from wt and CMT1A rats; these cultures replicate the "in vivo" setting obtained by exposing neurons to the same compound(s) as SC [Mithen et al., 1982; Melli and Höke, 2009]. DRG cultures were incubated for 4 weeks in the presence of 0.2 μM P18, replacing the medium with the compound and/or the vehicle every other day. The two structural isomers of P18, Ap2A, and P24 (both at 0.2 μM), were used as controls: P24 is a P2X7 agonist, whereas Ap2A is neither an agonist nor an antagonist of P2X7 [Bruzzone et al., 2010]; P24 and Ap2A also behave as P2Y11 agonists, inducing cAMP overproduction in P2Y11-overexpressing cells [Magnone et al., 2008]. The effect of the compounds on

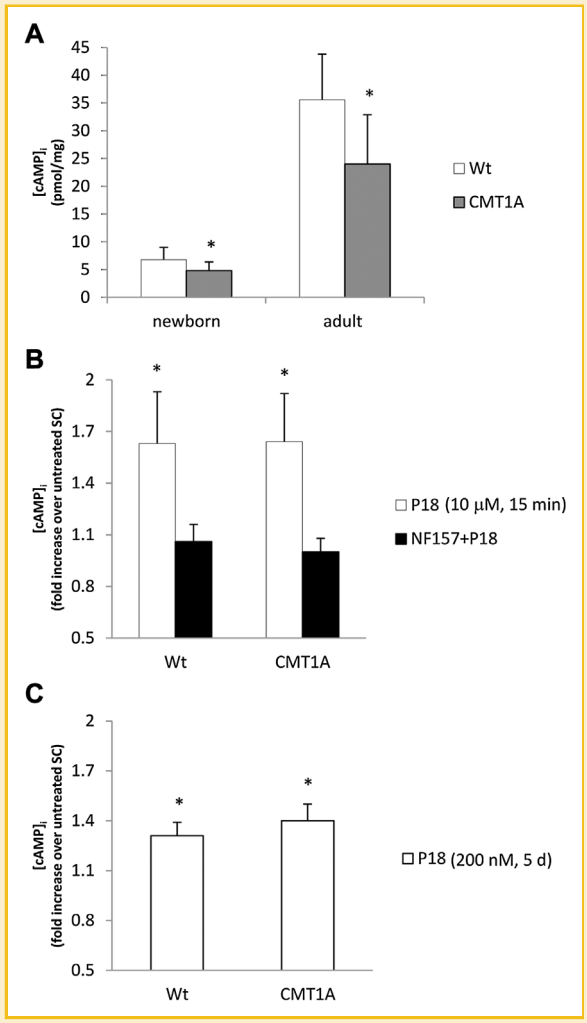

Fig. 1. The $[cAMP]_i$ levels are lower in CMT1A than in wt SC, and P18 increases $[cAMP]_i$ levels in wt and CMT1A SC. A: The $[cAMP]_i$ levels were measured in wt and CMT1A SC isolated from newborn (3 day-old, $n = 10$) or from adult (30 day-old, $n = 7$) rats. $^*P < 0.05$ compared to the corresponding wt. B: Wt and CMT1A SC were preincubated with or without 1 μM NF157 for 45 min and then treated (or not) for 15 min with 10 μM P18. Results are expressed as relative to untreated cells and are the mean ± SD of six determinations on different cultures. $^*P < 0.05$ compared to untreated SC. C: SC cultures were treated (or not) with 0.2 μM P18 for 5 days. At the end of each incubation, SC were lysed and the $[cAMP]_i$ was evaluated by ELISA assay. Results are expressed as relative to untreated cells and are the mean ± SD of six determinations on different SC cultures. $^*P < 0.05$ compared to untreated SC.

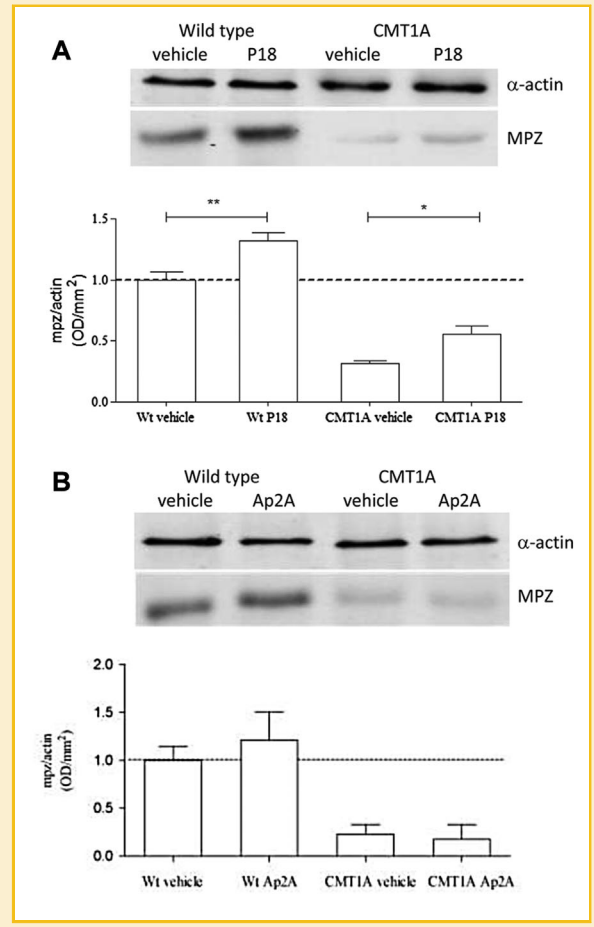

Fig. 2. Effect of P18 and Ap2A on MPZ expression in wt and CMT1A DRG cultures. Wt and CMT1A DRG cultures were treated or not (vehicle) for 4 weeks with 0.2 μM P18 (A) or Ap2A (B). MPZ expression was evaluated in DRG culture lysates by Western blot analysis and normalized to α-actin levels. Representative Western blots are shown; results in the graph indicate the mean ± SD from five experiments. Multiple-parameter comparisons were performed by one-way ANOVA followed by Bonferroni's post hoc test. $^*P < 0.05$; $^{**}P < 0.01$.

myelination was assessed by Western blot analysis of MPZ (Fig. 2), taken as a marker of myelin production. P18 significantly increased MPZ expression levels both in wt and in CMT1A cultures (Fig. 2A).

P24 proved to be toxic on DRG cultures, confirming earlier results on different cell lines [Basile et al., 2005; Bruzzone et al., 2007], including primary SC cultures [Bruzzone et al., 2010]. Ap2A did not induce any significant modification of MPZ synthesis, in either the wt or the CMT1A cultures (Fig. 2B).

Next, the positive effect of P18 on myelination was confirmed by immunohistochemistry on DRG cultures. As shown in Figure 3, morphologic examination of P18-treated CMT1A DRG cultures immunostained for MBP clearly showed a higher density of myelinated segments, in comparison with CMT1A DRG cultures (showing a similar density of NFs and nuclei) exposed to the vehicle alone. Conversely, P18 did not affect myelination, as evaluated by MBP immunostaining, in wt DRG cultures (Fig. 3).

Moreover, as axonal pathology almost invariably develops in CMT1A patients and axonal damage and loss are definitely present in CMT1A DRG cultures [Nobbio et al., 2006], we analyzed the effect of P18 on axonal features. The amount of non-phosphorylated NF, evaluated by Western blot analysis, was used to assess the degree of axonal damage [Nobbio et al., 2006]. Interestingly, P18 significantly reduced non-phosphorylated NF content only in pathological cultures, whereas it did not significantly affect non-phosphorylated NF content in wt cultures (Fig. 4). Chronic exposure to Ap2A did not modify the non-phosphorylated NF content either in CMT1A cultures or in the wt ones (not shown).

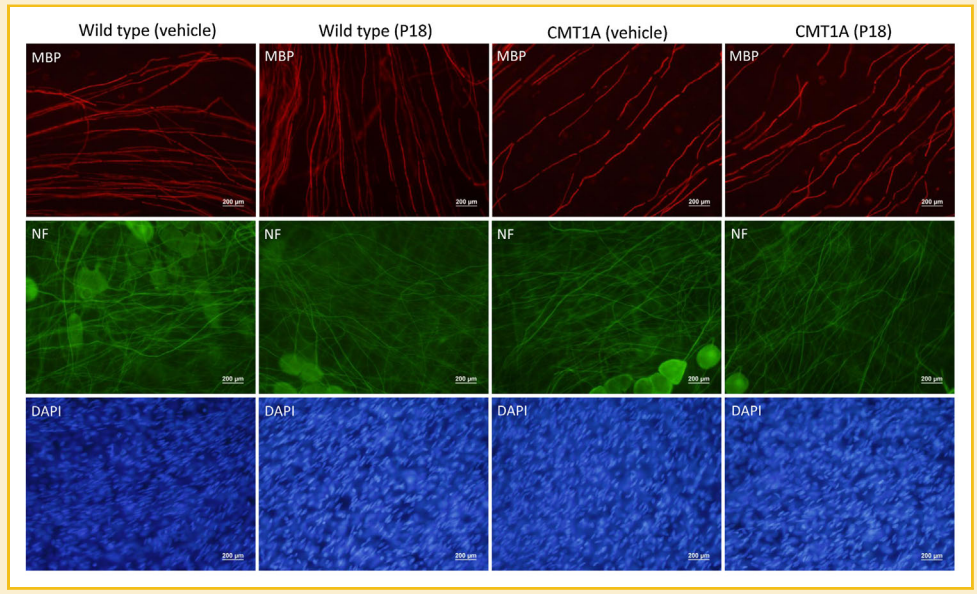

Fig. 3. Effect of P18 on myelination in wt and CMT1A DRG cultures. Wt and CMT1A DRG were allowed to myelinate for up to 4 weeks, in the presence (or absence, vehicle) of 0.2 μM P18. Immunocytochemistry for MBP on DRG cultures was then performed. Axons were stained with an anti-NF antibody and nuclei were stained with DAPI.

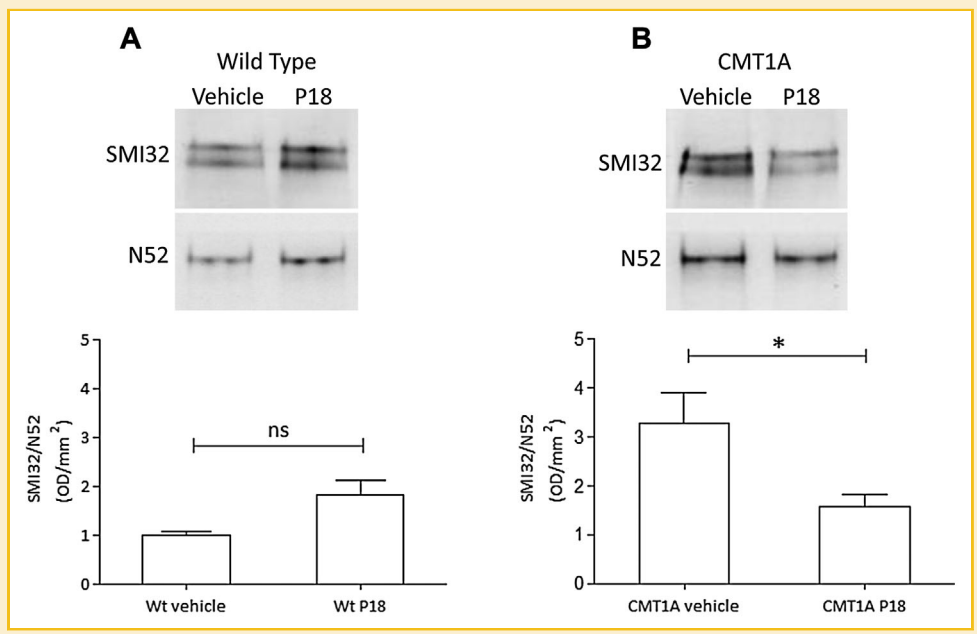

Fig. 4. P18 reduces non-phosphorylated NF content in CMT1A DRG cultures. Wt (A) and CMT1A (B) DRG cultures were treated for 4 weeks with 200 nM P18 and assessed by Western blot analysis for non-phosphorylated NF (SMI32) content or for total NF (N52). A representative Western blot analysis is shown. The amount of non-phosphorylated NF was expressed relative to total NF in the graph and results indicate the mean ± SD from eight determinations. ns, not significant; $^{*}P < 0.05$.

DISCUSSION

In our previous study, we reported that the P2X7 receptor is overexpressed in CMT1A SC, causing an abnormally high $[Ca^{2+}]_i$ in these cells. Correction of the elevated $[Ca^{2+}]_i$ levels restored normal migration, CNTF release, and in vitro myelinating capacity in CMT1A SC [Nobbio et al., 2009].

As myelination is subjected to both negative and positive controls [Jessen and Mirsky, 2008], and in CMT1A SC exhibiting a clear abnormal phenotype [Nobbio et al., 2004] we observed an

overerepresentation of negative myelin regulators as purinergic signaling (P2X7) and $[Ca^{2+}]_i$, we checked also for $[cAMP]_i$, due to increasing evidence pointing to cAMP as a key regulator of SC differentiation and function and peripheral myelination [Jessen et al., 1991; Monje et al., 2010; Guo et al., 2012]. Our results show significantly lower cAMP levels in primary cultures of CMT1A SC compared to the wt ones (Fig. 1), further supporting the impaired differentiation of these transgenic cells. Through experiments performed on a murine SC line (MSC80), it was suggested that PMP22 expression is controlled by the presence of a cAMP response element, which acts as a silencer in the absence of cAMP [Sabéran-Djoneidi et al., 2000]. AA treatment resulted in substantial amelioration of the CMT1A phenotype, and reduced the expression of PMP22 in a mouse model of CMT1A [Passage et al., 2004]. The AA effect was attributed to the reduced $[cAMP]_i$ content in AA-treated MSC80 cells, likely as a consequence of the AA-induced AC inhibition [Kaya et al., 2007]. Our data demonstrate that cAMP levels are in fact significantly lower in CMT1A SC than in wt cells, although *PMP22* gene is overexpressed in CMT1A SC. Thus, our results seem to rule out the possibility that endogenous cAMP acts as a physiological regulator of PMP22 expression in CMT1A SC.

We treated DRG cultures with P18, a newly identified P2X7 antagonist/P2Y11 agonist, capable of concomitantly reverting the $[Ca^{2+}]_i$ levels of CMT1A SC to normal [Bruzzone et al., 2010] and increasing their $[cAMP]_i$ (Fig. 1). Interestingly, P18 was effective in improving myelination, as assessed by Western blot analysis of MPZ levels and MBP staining (Figs. 2 and 3). Since P18 has antagonistic properties toward P2X7 but is also an agonist of P2Y11 which is the only P2 receptor known to mediate cAMP dependent signalling [Qi et al., 2001], we verified whether the beneficial effect of P18 in ameliorating myelination might be due, at least in part, to the P2Y11-mediated increase of cAMP levels. The fact that Ap2A, a P18 isomer which induces a P2Y11-mediated $[cAMP]_i$ increase (see Results Section), without modifying the $[Ca^{2+}]_i$ [Bruzzone et al., 2010], does not improve myelination, underscores the importance of P2X7 inhibition in the P18-induced effects in DRG cultures.

Interestingly, P18 impacts on axonal sufferance of CMT1A DRG cultures, as deduced from its lowering effect on non-phosphorylated NF (Fig. 4). The failure of Ap2A to lower non-phosphorylated NF in CMT1A cultures, despite its ability to increase the $[cAMP]_i$ (see above), demonstrates that the P18 effect in reducing non-phosphorylated NF content is not related to its ability to increase the $[cAMP]_i$ in SC.

The capacity of P18 to reduce axonal damage in CMT1A DRG cultures, suggests its possible utilization also for treatment of traumatic nerve injury and/or neuropathic pain, where involvement of P2X7 has indeed been already demonstrated, and to prevent or treat axonal damage in other demyelinating neuropathies [Fulgenzi et al., 2008; Perez-Medrano et al., 2009; Andó et al., 2010; d'Ydewalle et al., 2012].

In conclusion, our findings might suggest new therapeutic options for treatment of CMT1A, a condition for which no pathogenetic treatment is as yet available [Schenone et al., 2011; Li et al., 2013]. Recently, with a high-throughput approach [Jang et al., 2012], a number of drugs have been found to decrease PMP22 expression in culture, but none of them is applicable or ready for use in humans. Progesterone antagonists, which are effective in treating a rat model of CMT1A, have not yet been tested in human trials [Sereda et al., 2003; Meyer zu Horste et al., 2007; Schenone et al., 2011; Li et al., 2013]. AA, which held some promise to treat patients affected by this neuropathy, proved to be ineffective in several clinical trials [Pareyson et al., 2011].

Our results suggest that antagonizing the purinergic receptor P2X7, possibly in combination with $[cAMP]_i$-increasing agents, might represent a promising strategy for the therapy of CMT1A patients.

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