SHORT REPORT

Adrenomedullin promotes differentiation of oligodendrocyte precursor cells into myelin-basic-protein expressing oligodendrocytes under pathological conditions in vitro

Takakuni Maki [a], Yoko Takahashi [a,b], Nobukazu Miyamoto [a], Anna C. Liang [a], Masafumi Ihara [c], Eng H. Lo [a], Ken Arai [a,*]

[a] Neuroprotection Research Laboratory, Departments of Radiology and Neurology, MA General Hospital, Harvard Medical School, USA
[b] Department of Developmental Neuroscience, United Centers for Advanced Research and Translational Medicine, Tohoku University School of Medicine, Sendai, Japan
[c] Department of Stroke and Cerebrovascular Diseases, National Cerebral and Cardiovascular Center, Osaka, Japan

Abstract

Oligodendrocytes, which are the main cell type in cerebral white matter, are generated from their precursor cells (oligodendrocyte precursor cells: OPCs). However, the differentiation from OPCs to oligodendrocytes is disturbed under stressed conditions. Therefore, drugs that can improve oligodendrocyte regeneration may be effective for white matter-related diseases. Here we show that a vasoactive peptide adrenomedullin (AM) promotes the in vitro differentiation of OPCs under pathological conditions. Primary OPCs were prepared from neonatal rat brains, and differentiated into myelin-basic-protein expressing oligodendrocytes over time. This in vitro OPC differentiation was inhibited by prolonged chemical hypoxic stress induced by non-lethal $CoCl_2$ treatment. However, AM promoted the OPC differentiation under the hypoxic stress conditions, and the AM receptor antagonist AM_{22-52} canceled the AM-induced OPC differentiation. In addition, AM treatment increased the phosphorylation level of Akt in OPC cultures, and correspondingly, the PI3K/Akt inhibitor LY294002 blocked the AM-induced OPC differentiation. Taken together, AM treatment rescued OPC maturation under pathological conditions via an AM-receptor-PI3K/Akt pathway. Oligodendrocytes play critical roles in white matter by forming myelin sheath. Therefore, AM signaling may be a promising therapeutic target to boost oligodendrocyte regeneration in CNS disorders.

* Corresponding author at: Neuroprotection Research Laboratory, MGH East 149-2401, Charlestown, MA 02129, USA.
 E-mail address: karai@partners.org (K. Arai).

Introduction

Oligodendrocytes are one of the major cell types in the cerebral white matters. They produce a lipid-rich membrane called myelin, which enwrap up to 60 axonal segments each (i.e. myelin sheath). Myelin sheaths enable effective nerve impulse conduction and play a supporting role for axons and neuron homeostasis (Baumann and Pham-Dinh, 2001). During development, oligodendrocyte precursor cells (OPCs) are generated from germinal zones, then proliferate and migrate to both gray and white matter areas, where most of them differentiate into mature oligodendrocytes and form myelin sheaths. However, some OPCs may persist in an immature state. These residual OPCs are widely distributed in adult brains, comprising 5−8% of all cells (Levine et al., 2001). Although myelinated tracts are formed early in life, renewal of myelin and oligodendrocyte continues throughout most of the adult life (Paus et al., 1999; Young et al., 2013; Dimou et al., 2008). In fact, myelin sheaths in the adult CNS exhibit some plasticity in response to changes in neural activity (Scholz et al., 2009) and brain injury (Nait-Oumesmar et al., 2008). Residual OPCs may play an important part in these endogenous mechanisms of white matter repair and renewal.

In demyelinating diseases, injury of myelin/oligodendrocytes results in a profound loss of myelin sheaths, axonal injury and degeneration, which eventually lead to long-lasting functional disabilities (Franklin and Ffrench-Constant, 2008; Emery, 2010). Mouse models of oligodendrocyte injury, such as proteolipid protein (plp1)-null mice or Cnp mutant mice, show axon loss without considerable demyelination (Griffiths et al., 1998; Lappe-Siefke et al., 2003), suggesting that oligodendrocytes may also support axon survival through a myelin-independent mechanisms (Funfschilling et al., 2012). Endogenous repair attempts by OPC proliferation and differentiation would occur at the early stage of demyelinating disorders but often fail as disease progresses (Maki et al., 2013). Although no clinically proven agents currently exist to protect/support OPCs under prolonged or acute pathological conditions, enhancement of oligodendrogenesis (regeneration of mature myelinating oligodendrocytes) should be a promising approach for treatment of demyelinating diseases (Franklin and Ffrench-Constant, 2008; Kotter et al., 2011).

One potential target candidate for promoting oligodendrogenesis during pathological conditions may be adrenomedullin (AM). AM was discovered as a vasoactive peptide from human pheochromocytoma in 1993 (Kitamura et al., 1993; Kato et al., 2005). AM is widely distributed in tissues, and secreted from various organs such as adrenal medulla, heart, kidney, lung, and vascular wall as well as the brain. AM has diverse biological actions, including cell proliferation and differentiation in a paracrine and autocrine manner (Hinson et al., 2000; Martinez-Herrero et al., 2012; Lopez and Martinez, 2002; Shindo et al., 2013). AM also have important roles for cellular function of immature cells, such as endothelial progenitor cell (EPC), mesenchymal stem cell, hematopoietic stem cell, adrenocortical stem cell, and neural stem/progenitor cell (NSPC) (Martinez-Herrero et al., 2012; Larrayoz et al., 2012; Vergano-Vera et al., 2010). Recently, it has been reported that AM may also regulate white matter function in the brain. In a mouse model of white matter injury by prolonged cerebral hypoperfusion, increased levels of circulating AM preserved oligodendrocyte/myelin integrity along with restoring cerebral hemodynamic, promoting arteriogenesis/angiogenesis, and alleviating oxidative damage in cerebral microvessels (Maki et al., 2011a,b). In addition, AM deficiency exacerbated white matter injury during prolonged cerebral hypoperfusion conditions (Mitome-Mishima et al., 2014). However, mechanisms that underlie the ability of AM to protect myelin and oligodendrocytes in damaged white matter remain unclear. In this proof-of concept study, we tested the hypothesis that AM can promote OPC differentiation under pathological conditions.

Materials and methods

All experiments were performed following protocols approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Reagents

AM and AM receptor antagonists AM_{22-52} were purchased from Peptide Institute Inc., and dissolved in distilled water. $CoCl_2$ and LY294002 were purchased from Sigma, and dissolved in dimethysulphoxide. The final concentration of dimethysulphoxide in the culture medium was less than 0.1%, which had no effect on OPC survival and function.

Cell culture

OPCs were prepared as previously described (Arai and Lo, 2009; Pham et al., 2012). Briefly, cerebral cortices from 1−2 day old Sprague Dawley rats were dissected, minced, and digested. Dissociated cells were plated in poly-D-lysine-coated 75 cm² flasks, and maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 20% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. After the cells were confluent (~10 days), the flasks were shaken for 1 h on an orbital shaker (218 rpm) at 37 °C to remove microglia. They are then changed to new medium and shaken overnight (~20 h). The medium was collected and plated on non-coated tissue culture dishes for 1 h at 37 °C to eliminate contaminating astrocytes and microglia. The non-adherent cells were collected and replated in Neurobasal (NB) medium containing 2 mM glutamine, 1% penicillin/streptomycin, 10 ng/ml PDGF, 10 ng/ml FGF, and 2% B27 supplement onto poly-DL-ornithine-coated plates. Four to 5 days after plating, the OPCs were used for the experiments. To differentiate OPCs to oligodendrocytes, the culture medium was switched to DMEM containing 1% penicillin/streptomycin, 10 ng/ml CNTF, 15 nM T3, and 2% B27 supplement.

CoCl₂ treatment

To mimic hypoxic conditions in vitro, cells were incubated with non-lethal concentration of cobalt chloride ($CoCl_2$) according to our previous reports (Miyamoto et al., 2013a,b). For experiments in Figs. 1 and 3, cells were maintained in the differentiation culture media with or
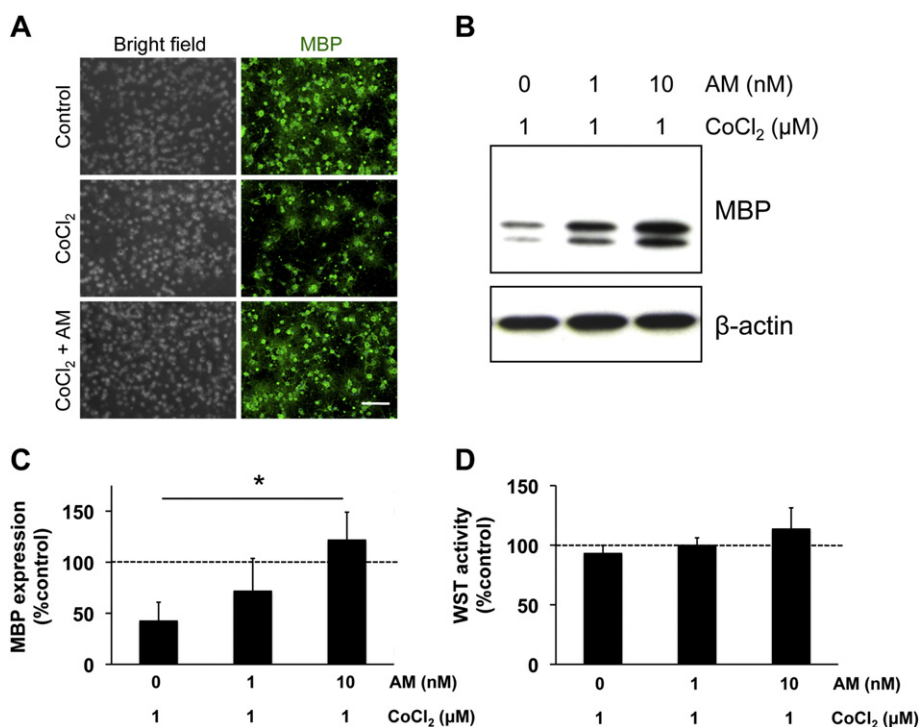
Figure 1 AM promoted OPC differentiation under prolonged hypoxic conditions. (A) Cultured rat OPCs were maintained in the differentiation media for 7 days. Under normal conditions, the OPCs were successfully matured into MBP-expressing oligodendrocytes. On the other hand, when OPCs were incubated with non-lethal 1 μM CoCl$_2$ (e.g. chemical hypoxic conditions), they were not differentiated. However, AM treatment (10 nM) promoted the in vitro OPC differentiation under the stressed conditions. MBP is a marker for mature oligodendrocytes. Scale bar = 200 nm. (B–C) Western blot analyses also confirmed that the hypoxic conditions disturbed the OPC differentiation but AM promoted the process. MBP is a marker for mature oligodendrocytes, and β-actin is an internal control. Percentages of MBP expression were calculated based on the values in control group (i.e. no CoCl$_2$ stress). Values are mean ± SD from 4 independent experiments. *P < 0.05. (D) The WST assay showed that either AM or CoCl$_2$ did not induce overt cell death in our OPC culture system. Percentages of WST activity were calculated based on the values in control group (i.e. no CoCl$_2$ stress). Values are mean ± SD from 4 independent experiments.

without 1 μM CoCl$_2$ for 7 days. Freshly prepared cultured media containing CoCl$_2$ were applied to OPC cultures on days 0, 3, and 5 (i.e. after starting OPC differentiation, we changed the culture media on days 3 and 5). Then on day 7 after starting OPC differentiation, cells were used for cell proliferation/survival assay, immunocytochemistry, and western blot. For experiments in Suppl Fig. S1, cells were maintained in the differentiation culture media for 7 days. We prepared four groups: (i) control (no CoCl$_2$ stress), (ii) 1 μM CoCl$_2$ (for 7 days), (iii) 1 μM CoCl$_2$ (for 7 days) plus 10 nM AM (for 7 days), and (iv) 1 μM CoCl$_2$ (for 7 days) plus 10 nM AM (from day 0 to day 1). After starting OPC differentiation, we changed the cultured media on days 1, 3, 5 and then on day 7, cells were used for western blot.

Cell proliferation/survival assay

Cell proliferation/survival was assessed by WST reduction assay (Cell Counting Kit-8, Dojindo). WST assay is a sensitive colorimetric method to detect cell viability. Highly water-soluble tetrazolium salt WST-8 is reduced by dehydrogenase activities in live cells to give a yellow-color formazan dye, which is soluble in the tissue culture media. Therefore, the intensity of the formazan dye can be an indicator correlating to the number of viable cells. The cells were incubated with 10% WST solution for 1 h at 37 °C. Then the absorbance of the culture medium was measured with a microplate reader at a test wavelength of 450 nm and a reference wavelength of 630 nm.

Immunocytochemistry

The cells were washed with ice-cold PBS, pH 7.4, followed by 4% paraformaldehyde for 15 min. After being further washed three times in PBS containing 0.1% Triton X-100, they were incubated with 1% bovine serum albumin in PBS for 1 h. Then cells were incubated with primary antibodies against MBP (1:100, Thermo Scientific) at 4 °C overnight. After washing with PBS, they were incubated with secondary antibodies for 1 h at room temperature. Finally, nuclei were counterstained with DAPI.

Western blotting

Cells were rinsed twice with ice-cold PBS, and the cells were collected into cell lysis buffer (Pro-PREP™ Protein Extraction Kit, iNtRON Biotechnology). After the protein concentrations were quantified and adjusted to the same concentrations by

adding PBS, samples were mixed with equal volumes of sample buffer containing 91% SDS (Novex) and 9% 2-mercaptoethanol (Sigma). Subsequently, samples were heated at 95 °C for 5 min, and each sample was loaded onto 4–20% Tris–glycine gels. After electrophoresis and transferring to nitrocellulose membranes (Novex), the membranes were blocked in Tris buffered saline with 0.1% Tween 20 (TBS-T) containing 5% nonfat dry milk for 60 min at room temperature. Membranes were then incubated overnight at 4 °C with myelin basic protein (MBP) antibody (1:500, Thermo Scientific), Akt antibody (1:1000, Cell Signaling), phosphorylated Akt (Ser473) antibody (1:1000, Cell Signaling), or anti-β-actin antibody (1:10000, Sigma Aldrich) followed by incubation with peroxidase-conjugated secondary antibodies and visualization by enhanced chemiluminescence (Amersham).

Statistical analysis

Experiments were performed in duplicate, repeated 3–4 times independently. Quantitative data were analyzed by using ANOVA followed by post hoc Tukey test or Tukey–Kramer test. All values are expressed as means ± SD. A value of $P < 0.05$ was considered statistically significant.

Results

Primary OPCs were prepared from rat neonatal cortex. When OPCs were maintained in differentiation media (e.g. DMEM plus 2% B27 including 10 ng/ml CNTF, 15 nM T3 over 7 days), the cells began to exhibit oligodendrocyte-like phenotypes with myelin-basic-protein (MBP) expression (Fig. 1A: control group). As previously reported, OPC differentiation was suppressed by prolonged hypoxia induced by non-lethal $CoCl_2$ treatment for 7 days (Miyamoto et al., 2013a,b) (Fig. 1A: $CoCl_2$-treated group). However, 7-day treatment with AM rescued OPC differentiation under the prolonged chemical hypoxic conditions (Fig. 1A: AM + $CoCl_2$-treated group). Western blot analyses confirmed that AM promoted oligodendrocyte maturation under the stressed conditions (Figs. 1B–C). However, 1-day AM treatment did not rescue the OPC differentiation under the stressed conditions (Suppl Fig. S1). A standard WST assay showed that neither AM nor $CoCl_2$ altered the OPC number or induced overt cell death in our cell culture system (Fig. 1D).

We next examined the mechanisms that may underlie AM-mediated rescue of OPC differentiation. Receptor-mediated Akt signaling may be involved since AM treatment increased the phosphorylation levels of Akt in OPCs within 30 min (Fig. 2A), and this Akt phosphorylation was back to baseline in 24 h after AM treatment (Fig. 2B). AM-induced Akt phosphorylation was inhibited by the PI3K inhibitor LY294002 (Fig. 2C) or blockade of the AM receptor with AM$_{22-52}$ (Fig. 2D). Finally, blockade of PI3K/Akt signaling with LY294002 or AM$_{22-52}$ negated the ability of AM to rescue OPC differentiation treatment under prolonged hypoxic conditions (Figs. 3A–B). These effects were not due to changes in cell survival since the WST assay once again showed that there was no significant cell damage in our experimental conditions (Fig. 3C).

Discussion

In this study, we obtained proof-of-concept data to support our hypothesis that AM can rescue OPC differentiation into mature oligodendrocytes under pathological conditions. Our pharmacological approaches also showed that the AM receptor and PI3K/Akt would mediate these AM effects. AM and its receptors are widely expressed in the central nervous system (CNS) (Ueta et al., 1995; Serrano et al., 2002). Past studies extensively examined the multiple roles of AM on neuronal and vascular function. AM exerts various actions on the vasculature, such as vasodilation, angiogenesis, and regulation of blood brain barrier. Similarly, AM acts as a neurotransmitter, neuromodulator, or neurohormone (Martinez-Herrero et al., 2012). In addition, AM can be considered as a therapeutic target for CNS diseases since several animal studies have demonstrated that AM reduces neuronal injuries (Watanabe et al., 2001; Miyashita et al., 2006; Xia et al., 2004, 2006). Furthermore, compared to wild-type mice, brain-specific conditional AM knockout mice or AM heterozygous KO mice exhibited more neuronal damage after ischemic insults (Hurtado et al., 2010; Miyamoto et al., 2009). In vitro cell culture studies also confirmed that AM protected neurons against oxygen glucose deprivation stress in an autocrine and paracrine manner (Miyashita et al., 2006; Tixier et al., 2008). AM may also be effective in the chronic phase as AM increased mobilization of CD34+ mononuclear cells (so-called EPCs) and subsequent vascular regeneration and neurogenesis after stroke (Miyashita et al., 2006). Our current findings that AM can promote oligodendrogenesis under pathological conditions may support these past studies and confirm that the AM signaling would be the therapeutic target for neurological disorders, especially for white matter-related diseases.

Neurons play the central role in the brain, and therefore, neuroprotection would be the most important approach for CNS diseases. However, oligodendrocytes (and oligodendrocyte-rich white matter) should also be considered when we aim to develop efficient therapies for brain protection. Compared to rodents, primates possess an evolutionally expanded volume of white matter, and white matter damage is a clinically important aspect of several CNS diseases, such as stroke or vascular dementia (Benowitz and Carmichael, 2010; Jiang et al., 2010; Yang and Rosenberg, 2011; Tosto et al., 2014; Philips and Rothstein, 2014). This may explain the reasons why many neuroprotectants (e.g. glutamate receptor antagonists, antioxidants, etc.) that were proved neuroprotective in rodent CNS disease models have failed to provide efficacy in clinical trials (Savitz and Fisher, 2007). Even small lesions in the white matter areas (corona radiata or internal capsule) could lead to severe hemiplegia and poor functional prognosis in humans because loss of oligodendroglial supports can cause progressive axon/neuron degeneration and long-term functional disability. Others and we have previously demonstrated that AM might play an important role in the preservation of oligodendrocyte and white matter integrity in mouse models of white matter injury (Maki et al., 2011a,b; Mitome-Mishima et al., 2014). For example, overexpression of circulating AM increased GST-pi-positive oligodendrocytes and preserved myelin integrity accompanied with promotion of neovascularization and vasoprotection after prolonged cerebral hypoperfusion in mice. This "oligo-vascular" protection may lead to the
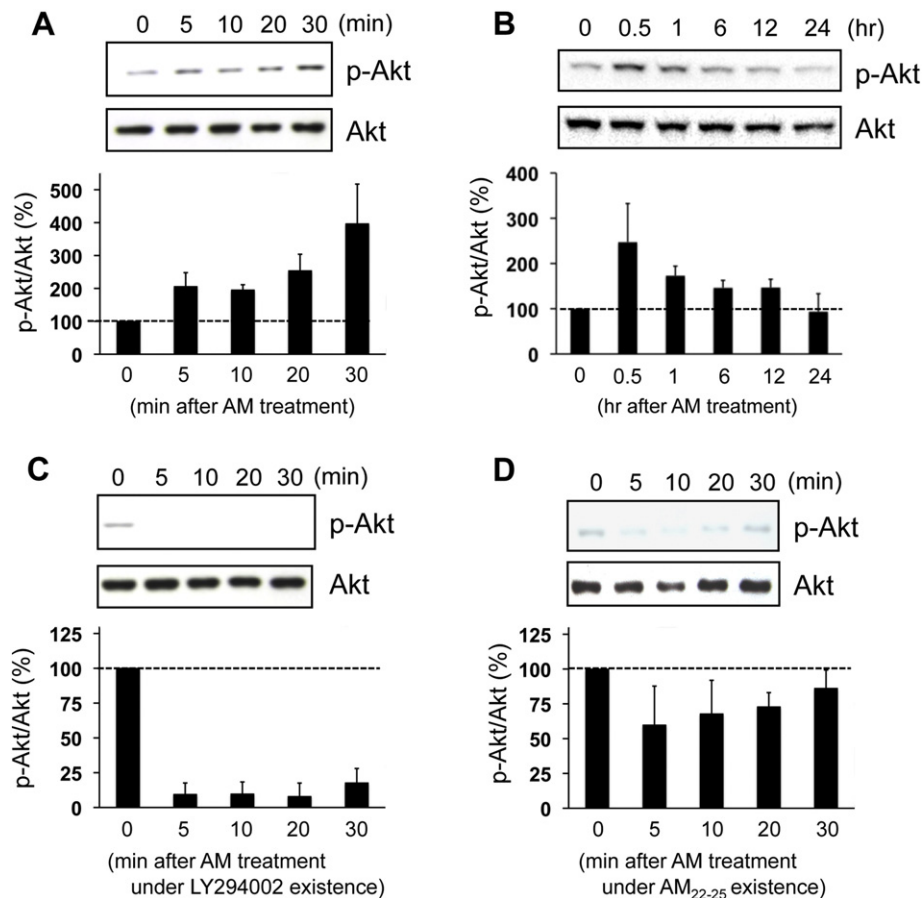
Figure 2 AM increased the levels of Akt phosphorylation via AM-receptor-PI3K pathway. (A–B) Cultured rat OPCs were incubated with 10 nM AM up to 24 h, and then cell lysates were subjected to western blot analyses with anti-p-Akt or anti-Akt antibodies. AM treatment increased the levels of Akt phosphorylation without changing total Akt levels. Values are mean ± SD from 3 independent experiments. (C) Under the conditions of 1 μM LY294002 (PI3K inhibitor) existence, AM did not increase the phosphorylation levels of Akt. Values are mean ± SD from 3 independent experiments. (D) Similarly, AM did not increase the phosphorylation levels of Akt under the conditions of 1 μM AM$_{22-52}$ (AM-receptor antagonist) existence. Values are mean ± SD from 3 independent experiments.

prevention of cognitive decline after demyelination (Maki et al., 2011a,b). In addition, a recent report showed that AM knockout mice exhibited decreased OPCs and GST-pi-positive oligodendrocytes and MBP expression in white matter after prolonged cerebral hypoperfusion (Mitome-Mishima et al., 2014). Here we show for the first time that AM would directly work on OPCs to promote oligodendrogenesis under pathological conditions in vitro. These findings may explain the mechanisms for beneficial effects of AM on white matter integrity and function. Therefore, the multiple actions of AM on neuro-vascular-oligo protection/regeneration would have a potential as a promising treatment for cerebrovascular diseases.

Taken together, our findings support the hypothesis that AM can rescue OPC differentiation via receptor-mediated Akt signaling. However, there are some important caveats to keep in mind. First, our current study used only a pure cell culture system. However, to prove clinically-relevant supportive/protective roles of AM on OPCs against stress, we should test the efficacy of AM on in vivo white matter injury animals. Second, our data indicate that short-term AM treatment was not supportive for in vitro OPC differentiation under pathological conditions. A single treatment of

AM could activate the downstream pathway (i.e. Akt phosphorylation), but to sufficiently drive the OPC differentiation, multiple rounds of AM treatments would be required. Before testing the efficacy of AM using in vivo animal models, further investigation into the underlying mechanisms of AM/Akt-induced OPC maturation is needed to identify effective treatment schedules of AM. Third, we only examined the PI3K/Akt pathway as an intracellular signaling pathway for in vitro oligodendrogenesis by AM. But the AM receptor would activate other cellular signaling pathways, such as MEK/ERK or cAMP/PKA pathways (Nagaya et al., 2005). Whether these pathways are also involved in the OPC-supportive effects of AM should be carefully examined in future studies. Finally, as OPCs are generated from NSPCs, we may also need to test if AM can enhance the number of newly generated OPCs from NSPCs after white matter injury. A recent study showed that lack of AM results in profound changes in the proliferation and differentiation rates in the progeny of NSPCs isolated from the olfactory bulbs of AM deficient mice. NSPCs derived from the AM deficient mice produced a lower proportion of neuronal-astroglial lineage cells and a higher proportions of oligodendrocyte lineage cells compared to NSPCs from WT mice (Vergano-Vera et al., 2010). Hence, future studies are
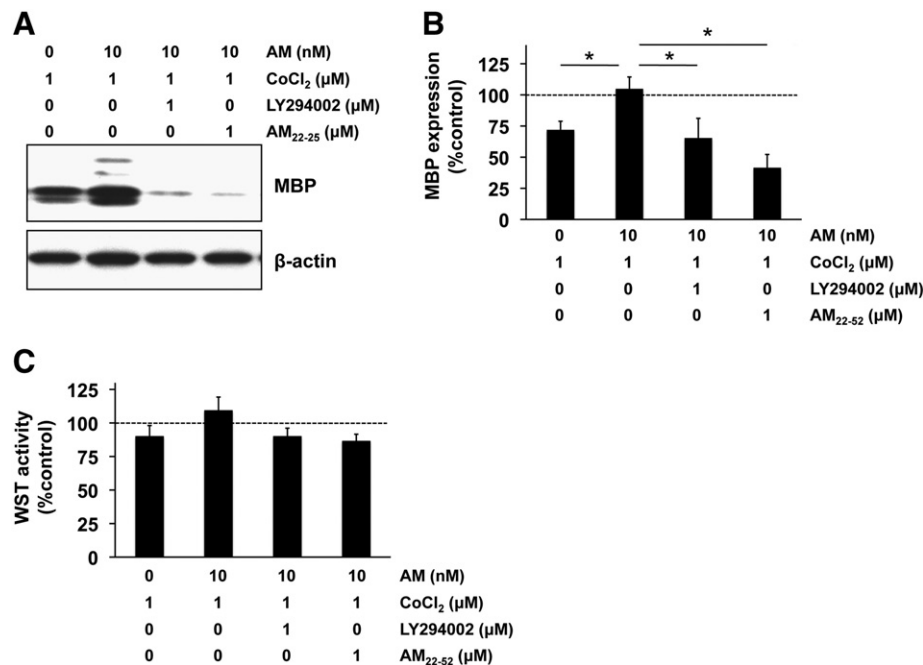
Figure 3 AM-receptor-PI3K/Akt pathway mediated AM-induced OPC maturation. (A−B) Cultured rat OPCs were maintained in the differentiation media for 7 days. The prolonged chemical hypoxic conditions by non-lethal 1 μM $CoCl_2$ disturbed OPC maturation, but AM treatment promoted the process. This AM-induced OPC maturation was inhibited by either co-treatment with 1 μM LY294002 (PI3K inhibitor) or 1 μM AM_{22-52} (AM-receptor antagonist). Percentages of MBP expression were calculated based on the values in control group (i.e. no $CoCl_2$ stress). N = 3 of independent experiments. *P < 0.05. (B) The WST assay showed that in any treatment groups, there was no significant OPC damage. Percentages of WST activity were calculated based on the values in control group (i.e. no $CoCl_2$ stress). Values are mean ± SD from 3 independent experiments.

warranted to examine how AM regulates the cell fates of NSPCs under normal and pathological conditions.

In summary, our data provide proof-of-concept that AM can promote and rescue OPC differentiation into mature oligodendrocytes under pathological conditions in vitro. Preservation and repair of oligodendrocytes should be an important criteria of therapies for CNS disease patients. Therefore, AM signaling may be a novel therapeutic target for accelerating regenerative responses in demyelinating conditions such as stroke, multiple sclerosis or vascular dementia.

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References

Arai, K., Lo, E.H., 2009. An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. J. Neurosci. 29, 4351–4355.

Baumann, N., Pham-Dinh, D., 2001. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol. Rev. 81, 871–927.

Benowitz, L.I., Carmichael, S.T., 2010. Promoting axonal rewiring to improve outcome after stroke. Neurobiol. Dis. 37, 259–266.

Dimou, L., Simon, C., Kirchhoff, F., Takebayashi, H., Gotz, M., 2008. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J. Neurosci. 28, 10434–10442.

Emery, B., 2010. Regulation of oligodendrocyte differentiation and myelination. Science 330, 779–782.

Franklin, R.J., Ffrench-Constant, C., 2008. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855.

Funfschilling, U., Supplie, L.M., Mahad, D., Boretius, S., Saab, A.S., Edgar, J., Brinkmann, B.G., Kassmann, C.M., Tzvetanova, I.D., Mobius, W., Diaz, F., Meijer, D., Suter, U., Hamprecht, B., Sereda, M.W., Moraes, C.T., Frahm, J., Goebbels, S., Nave, K.A., 2012. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485, 517–521.

Griffiths, I., Klugmann, M., Anderson, T., Yool, D., Thomson, C., Schwab, M.H., Schneider, A., Zimmermann, F., McCulloch, M., Nadon, N., Nave, K.A., 1998. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280, 1610–1613.

Hinson, J.P., Kapas, S., Smith, D.M., 2000. Adrenomedullin, a multifunctional regulatory peptide. Endocr. Rev. 21, 138–167.

Hurtado, O., Serrano, J., Sobrado, M., Fernandez, A.P., Lizasoain, I., Martinez-Murillo, R., Moro, M.A., Martinez, A., 2010. Lack of adrenomedullin, but not complement factor H, results in larger infarct size and more extensive brain damage in a focal ischemia model. Neuroscience 171, 885–892.

Jiang, Q., Zhang, Z.G., Chopp, M., 2010. MRI evaluation of white matter recovery after brain injury. Stroke 41, S112–S113.

Kato, J., Tsuruda, T., Kita, T., Kitamura, K., Eto, T., 2005. Adrenomedullin: a protective factor for blood vessels. Arterioscler. Thromb. Vasc. Biol. 25, 2480–2487.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kotter, M.R., Stadelmann, C., Hartung, H.P., 2011. Enhancing remyelination in disease—can we wrap it up? Brain 134, 1882–1900.

Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P.E., Griffiths, I.R., Nave, K.A., 2003. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat. Genet. 33, 366–374.

Larrayoz, I.M., Ochoa-Callejero, L., Garcia-Sanmartin, J., Vicario-Abejon, C., Martinez, A., 2012. Role of adrenomedullin in the growth and differentiation of stem and progenitor cells. Int. Rev. Cell Mol. Biol. 297, 175–234.

Levine, J.M., Reynolds, R., Fawcett, J.W., 2001. The oligodendrocyte precursor cell in health and disease. Trends Neurosci. 24, 39–47.

Lopez, J., Martinez, A., 2002. Cell and molecular biology of the multifunctional peptide, adrenomedullin. Int. Rev. Cytol. 221, 1–92.

Maki, T., Ihara, M., Fujita, Y., Nambu, T., Miyashita, K., Yamada, M., Washida, K., Nishio, K., Ito, H., Harada, H., Yokoi, H., Arai, H., Itoh, H., Nakao, K., Takahashi, R., Tomimoto, H., 2011a. Angiogenic and vasoprotective effects of adrenomedullin on prevention of cognitive decline after chronic cerebral hypoperfusion in mice. Stroke 42, 1122–1128.

Maki, T., Ihara, M., Fujita, Y., Nambu, T., Harada, H., Ito, H., Nakao, K., Tomimoto, H., Takahashi, R., 2011b. Angiogenic roles of adrenomedullin through vascular endothelial growth factor induction. Neuroreport 22, 442–447.

Maki, T., Hayakawa, K., Pham, L.D., Xing, C., Lo, E.H., Arai, K., 2013. Biphasic mechanisms of neurovascular unit injury and protection in CNS diseases. CNS Neurol. Disord. Drug Targets 12, 302–315.

Martinez-Herrero, S., Larrayoz, I.M., Ochoa-Callejero, L., Garcia-Sanmartin, J., Martinez, A., 2012. Adrenomedullin as a growth and cell fate regulatory factor for adult neural stem cells. Stem Cells Int. 2012, 804717.

Mitome-Mishima, Y., Miyamoto, N., Tanaka, R., Shimosawa, T., Oishi, H., Arai, H., Hattori, N., Urabe, T., 2014. Adrenomedullin deficiency and aging exacerbate ischemic white matter injury after prolonged cerebral hypoperfusion in mice. BioMed Res. Int. 2014, 861632.

Miyamoto, N., Tanaka, R., Shimosawa, T., Yatomi, Y., Fujita, T., Hattori, N., Urabe, T., 2009. Protein kinase A-dependent suppression of reactive oxygen species in transient focal ischemia in adrenomedullin-deficient mice. J. Cereb. Blood Flow Metab. 29, 1769–1779.

Miyamoto, N., Maki, T., Pham, L.D., Hayakawa, K., Seo, J.H., Mandeville, E.T., Mandeville, J.B., Kim, K.W., Lo, E.H., Arai, K., 2013a. Oxidative stress interferes with white matter renewal after prolonged cerebral hypoperfusion in mice. Stroke 44, 3516–3521.

Miyamoto, N., Pham, L.D., Hayakawa, K., Matsuzaki, T., Seo, J.H., Magnain, C., Ayata, C., Kim, K.W., Boas, D., Lo, E.H., Arai, K., 2013b. Age-related decline in oligodendrogenesis retards white matter repair in mice. Stroke 44, 2573–2578.

Miyashita, K., Itoh, H., Arai, H., Suganami, T., Sawada, N., Fukunaga, Y., Sone, M., Yamahara, K., Yurugi-Kobayashi, T., Park, K., Oyamada, N., Sawada, N., Taura, D., Tsujimoto, H., Chao, T.H., Tamura, N., Mukoyama, M., Nakao, K., 2006. The neuroprotective and vasculo-neuro-regenerative roles of adrenomedullin in ischemic brain and its therapeutic potential. Endocrinology 147, 1642–1653.

Nagaya, N., Mori, H., Murakami, S., Kangawa, K., Kitamura, S., 2005. Adrenomedullin: angiogenesis and gene therapy. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1432–R1437.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C., Baron-Van Evercooren, A., 2008. The role of SVZ-derived neural precursors in demyelinating diseases: from animal models to multiple sclerosis. J. Neurol. Sci. 265, 26–31.

Paus, T., Zijdenbos, A., Worsley, K., Collins, D.L., Blumenthal, J., Giedd, J.N., Rapoport, J.L., Evans, A.C., 1999. Structural maturation of neural pathways in children and adolescents: in vivo study. Science 283, 1908–1911.

Pham, L.D., Hayakawa, K., Seo, J.H., Nguyen, M.N., Som, A.T., Lee, B.J., Guo, S., Kim, K.W., Lo, E.H., Arai, K., 2012. Crosstalk between oligodendrocytes and cerebral endothelium contributes to vascular remodeling after white matter injury. Glia 60, 875–881.

Philips, T., Rothstein, J.D., 2014. Glial cells in amyotrophic lateral sclerosis. Exp. Neurol. 262, 111–120.

Savitz, S.I., Fisher, M., 2007. Future of neuroprotection for acute stroke: in the aftermath of the SAINT trials. Ann. Neurol. 61, 396–402.

Scholz, J., Klein, M.C., Behrens, T.E., Johansen-Berg, H., 2009. Training induces changes in white-matter architecture. Nat. Neurosci. 12, 1370–1371.

Serrano, J., Alonso, D., Fernandez, A.P., Encinas, J.M., Lopez, J.C., Castro-Blanco, S., Fernandez-Vizarra, P., Richart, A., Santacana, M., Uttenthal, L.O., Bentura, M.L., Martinez-Murillo, R., Martinez, A., Cuttitta, F., Rodrigo, J., 2002. Adrenomedullin in the central nervous system. Microsc. Res. Tech. 57, 76–90.

Shindo, T., Sakurai, T., Kamiyoshi, A., Ichikawa-Shindo, Y., Shimoyama, N., Iinuma, N., Arai, T., Miyagawa, S., 2013. Regulation of adrenomedullin and its family peptide by RAMP system—lessons from genetically engineered mice. Curr. Protein Pept. Sci. 14, 347–357.

Tixier, E., Leconte, C., Touzani, O., Roussel, S., Petit, E., Bernaudin, M., 2008. Adrenomedullin protects neurons against oxygen glucose deprivation stress in an autocrine and paracrine manner. J. Neurochem. 106, 1388–1403.

Tosto, G., Zimmerman, M.E., Carmichael, O.T., Brickman, A.M., 2014. Predicting aggressive decline in mild cognitive impairment: the importance of white matter hyperintensities. JAMA Neurol. 71, 872–877.

Ueta, Y., Kitamura, K., Isse, T., Shibuya, I., Kabashima, N., Yamamoto, S., Kangawa, K., Matsuo, H., Eto, T., Yamashita, H., 1995. Adrenomedullin-immunoreactive neurons in the paraventricular and supraoptic nuclei of the rat. Neurosci. Lett. 202, 37–40.

Vergano-Vera, E., Fernandez, A.P., Hurtado-Chong, A., Vicario-Abejon, C., Martinez, A., 2010. Lack of adrenomedullin affects growth and differentiation of adult neural stem/progenitor cells. Cell Tissue Res. 340, 1–11.

Watanabe, K., Takayasu, M., Noda, A., Hara, M., Takagi, T., Suzuki, Y., Yoshia, J., 2001. Adrenomedullin reduces ischemic brain injury after transient middle cerebral artery occlusion in rats. Acta Neurochir. 143, 1157–1161.

Xia, C.F., Yin, H., Borlongan, C.V., Chao, J., Chao, L., 2004. Adrenomedullin gene delivery protects against cerebral ischemic injury by promoting astrocyte migration and survival. Hum. Gene Ther. 15, 1243–1254.

Xia, C.F., Yin, H., Borlongan, C.V., Chao, J., Chao, L., 2006. Postischemic infusion of adrenomedullin protects against ischemic stroke by inhibiting apoptosis and promoting angiogenesis. Exp. Neurol. 197, 521–530.

Yang, Y., Rosenberg, G.A., 2011. Blood–brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke 42, 3323–3328.

Young, K.M., Psachoulia, K., Tripathi, R.B., Dunn, S.J., Cossell, L., Attwell, D., Tohyama, K., Richardson, W.D., 2013. Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron 77, 873–885.