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Rolipram promotes remyelination possibly via MEK-ERK signal pathway in cuprizone-induced demyelination mouse

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ABSTRACT

Objective: Rolipram, a 3′–5′-cyclic adenosine monophosphate (cAMP)-dependent phosphodiesterase 4 (PDE4) inhibitor, has long been studied for its immune modulating effects in the treatment of experimental autoimmune encephalomyelitis (EAE). In the current study, we investigated the effects of rolipram on remyelination after cuprizone- or lysolecithin-induced demyelination and the signal transduction pathways potentially modulating this response.

Materials and methods: Cuprizone-induced demyelination in mice and lysolecithin (LPC)-induced demyelination in rat cerebellum slice culture were treated with rolipram. Demyelination was evaluated by Luxol fast blue (LFB) or myelin basic protein (MBP) staining and western blot. Oligodendroglial cells were cultured with different concentrations of rolipram, and 2′, 3′-cyclic nucleotide phosphodiesterase (CNPase) activity, MBP expression, and extracellular signal-regulated kinase (ERK) phosphorylation were measured.

Results: Rolipram antagonized lysolecithin (LPC)-induced demyelination in rat cerebellar slice cultures and cuprizone-fed mice. In vitro, rolipram treatment promoted oligodendrocyte precursor cell (OPC) maturation, an effect that was partially blocked by the inhibitors of the mitogen activated protein kinase kinase (MEK).

Conclusion: Rolipram promotes the maturation of OPCs, facilitates remyelination, and increases ERK phosphorylation. All of these actions are involved in an action against cuprizone-induced demyelination that may occur partly via the MEK-ERK pathway. Importantly, this may have therapeutic implications for MS.

Introduction

Multiple sclerosis (MS) is a chronic CNS autoimmune disease characterized by an inappropriateTh1 response (Weiner, 2009). Demyelination is one of the probable pathological causes of the edema and axon loss associated with MS. However, demyelination may be followed by a spontaneous regenerative remyelination that has been shown in animal models to be dependent on the recruitment and differentiation of oligodendrocyte precursor cells (OPCs) (Chang et al., 2002). A common cause of remyelination failure in MS patients is not an absence of OPCs (these are often present in abundance) but a failure of OPCs to differentiate into remyelinating oligodendrocytes (Chang et al., 2002; Kuhlmann, et al., 2008; Wolswijk, 1998).

Over the past few decades, statins (HMGCoA reductase inhibitors) have been used for their immunomodulatory characteristics, including causing a shift from Th1 to Th2 immune responses in autoimmune diseases such as MS (Aktas et al., 2003; Youssef et al., 2002). Phosphodiesterase 4 (PDE4) inhibitors, such as rolipram, also shift the immune response from a Th1 to a Th2 response (Sommer et al., 1995), and have been used in experimental autoimmune encephalitis (EAE), an animal model of MS (Bielekova et al., 2000). Studies with rolipram in EAE in mice, rats, and nonhuman primates have demonstrated its efficacy in this disease model, suggesting that a PDE4 inhibitor such as rolipram might also be useful in the treatment of MS (Bielekova et al., 2009; Folcik et al., 1999; Genain et al., 1995; Martinez et al., 1999).

However inflammation-mediated demyelination is not the only process driving MS, for although drugs that act against the immune system are effective in the early, relapsing–recurring stage of MS, they are ineffective against the later insidious-progressive stage of this disease (Aktas et al., 2009; Dutta and Trapp, 2011). PDE4, a primary enzyme for metabolizing cAMP, is found in oligodendrocytes as well as immune cells (Whitaker et al., 2008). A recent study demonstrated that the combined treatment of suboptimal doses of lovastatin and rolipram decreases the severity of EAE by decreasing inflammation, axonal loss, and demyelination (Paintlia et al., 2008), and the authors suggested that this combination had the potential for use in MS (Paintlia et al., 2009). It is possible that the therapeutic

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effect of rolipram comes from inhibition of PDE4 and subsequent increase in cAMP in oligodendrocytes as well as from inhibition of this enzyme in immune cells and shift from a Th1 to a Th2 immune response. In neurons, elevated levels of cAMP are reported to promote axonal growth even in the presence of myelin-associated inhibitors of regeneration (Domeniconi and Filbin, 2005).

The extracellular signal receptor kinase (ERK) phosphorylation cascade, one of four mitogen-activated protein kinase (MAPK) signaling pathways, is important in oligodendrocyte differentiation (Fyffe-Maricich et al., 2011). Loss of ERK causes a decrease in the number of OPCs (Newbern et al., 2011) and treatment of oligodendroglial cells with a MAPK kinase inhibitor causes a decrease in the number of mature oligodendrocytes (Younes-Rapozo et al., 2009). The up-regulation of ERK plays a critical role in myelination. An increase in cAMP can cause phosphorylation and activation of ERK, and the increase in cAMP caused by rolipram's inhibition of its breakdown might be a second mechanism by which rolipram treatment could affect myelination.

In the present study, we evaluated the potential role of rolipram in neurorepair in cell culture, brain slices, and in vivo. In primary oligodendroglial cultures, we showed that rolipram promoted OPC maturation, possibly partly through the induction of ERK phosphorylation. We also studied rolipram's role in neurorepair in two toxic demyelination models, lysolecithin (LPC)-induced demyelination in cerebellum slice culture and cuprizone-induced demyelination in mice. We show for the first time that rolipram promotes remyelination after cuprizone-induced demyelination in vivo and in LPC-treated cerebellar slice cultures.

Materials and methods

Animals

C57BL/6 mice aged 6–8 weeks, neonatal Sprague–Dawley (SD) rats, and 10 day postnatal SD rats were purchased from Shanghai Laboratory Animal Center. Male mice were randomized into 4 different groups, including 2 groups for therapeutic study and 2 groups for preventive study with 8–9 treatment mice and 3 untreated control mice in each group. Animals underwent routine cage maintenance once a week. Food and water were available ad libitum. All research and animal care procedures were approved by guidelines of the Animal Care and Use Committee in Harbin Medical University. Procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications No.80-23, revised 1996).

Antibodies and reagents

Antibodies for MBP and 2′3′ cyclic nucleotide 3′ phosphodiesterase (CNPase) were from Millipore Corporation (Billerica, MA). Antibody for myelin associated glycoprotein (MAG) was from Chemicon (Temecula, CA), and antibody for phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) was from Cell Signaling Technology (Beverley, MA). Alexa Fluor 488 donkey anti-mouse IgG was from Invitrogen (Carlsbad, CA). The MEK inhibitor U0126 was from Tocris Bioscience (Bristol, UK). Dulbecco's modified Eagle Medium (DMEM) and NuPAGE 4-12% Bis-Tris gels were from Invitrogen (Carlsbad, CA). All other antibodies and reagents were from Sigma Aldrich (St. Louis, MO).

Primary oligodendroglial cultures

Primary oligodendroglial cultures from neonatal SD rats were prepared as reported previously (Chen et al., 2007). Briefly, brains were extracted and the cortices removed and incubated in 0.05% trypsin/EDTA for 15 min at 37 °C. Cells were then plated in DMEM supplemented with 20% FBS. Mixed glia cultures were grown for 10 days, and oligodendrocytes were then separated by shaking the flasks overnight at 37 °C at 200 rpm. Oligodendrocytes were obtained via differential adhesion on nontissue culture-treated plates for 30 min. The cells were identified by morphology and plated on poly-D,L-ornithine-coated petri dishes in OPC medium (DMEM, 4 mM L-glutamine, 1 mM sodium pyruvate, 0.1%BSA,10 nM biotin, 10 nM hydrocortisone,10 ng/ml PDGF-AA and 10 ng/ml bFGF) for 4–7 days before being subjected to the differentiation assay.

Differentiation assay

OPCs were immediately separated and identified by morphology. The purity of the OPC preparation was greater than 95%. Contaminating microglia and astrocytes were less than 2 to 3%, respectively. Pure OPCs were seeded in poly-D,L-ornithine coated 96-well plates at 10^4/well with OPC medium. Rolipram-free DMSO was used as a negative control and triiodothyronine (T3, 40 ng/ml), a hormone known to induce oligodendrocyte differentiation (Baas et al., 2002), as a positive control. Different concentrations of rolipram were co-cultured with cells for 72 h before cells were fixed with 4% paraformaldeyde and stained for MBP and CNPase. Serum-free DMEM (with 4 mM L-glutamine, 1 mM sodium pyruvate, 0.1%BSA, 10 nM biotin, and 10 nM hydrocortisone) was used during rolipram treatment. Alexa Fluor 488 donkey anti-mouse IgG was used as secondary antibody. Cells were then subjected to a High Content Imager (TTP Labtech Acumen eX3) according to the manufacturer's instructions. For the MEK inhibition assay, similar procedures were performed. However, OPCs were seeded in 384-well plates at 3000/well with OPC medium, co-cultured with 5 μM rolipram and different doses of U0126. They were then subjected to Cellomics Array Scan VTI High Content Imager (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. All assays were performed in triplicate.

Slice culture

Slice cultures were prepared as previously described (Birgbauer et al., 2004; Stoppini et al., 1991). Postnatal day 10 SD rats were decapitated and the cerebellums immediately isolated and transferred to a vibrating blade microtome (Leica VT1000S) that cut sagittal slices at a thickness of 300 μm. Every sixth to eighth slice was transferred to a 6-well membrane insert (Millipore-CM) with a pore size of 0.4 μm.The culture medium consisted of minimum essential medium (50%), Hank's balanced salt solution (25%), horse serum (25%), and 5 mg/ml glucose. For demyelination, medium was removed after 7 days in vitro and fresh medium supplemented with 0.5 mg/ml LPC was added. The slices were then incubated overnight (15–17 h) at 37 °C. After incubation, the LPC-containing medium was removed and replaced with fresh medium for 72 h. Rolipram (0.5 μM) was administered to the slices 1 h before LPC treatment and remained in the medium following LPC withdrawal. Lysates of slices were subjected to western blot and CNPase activity assay. For immune staining, whole mount staining was carried out as previously described (Birgbauer et al., 2004) with the use of MBP antibody and Alexa Fluor 488 donkey anti-mouse IgG.

CNPase activity assay

CNPase activity was measured using cNADP as substrate (Lee et al., 2001). This assay measures the rate of hydrolysis of cNADP to NADP, which is coupled to the dehydrogenation of glucose 6-phosphate catalyzed by glucose-6-phosphate dehydrogenase. Briefly, the assay mixture (1 ml) consisted of 100 mM MES, pH 6.0, 30 mM MgCl2, 5 mM D-glucose 6-phosphate, 5 μg D-glucose-6-phosphate dehydrogenase, and 2.5 mM cNADP. After the addition of CNP to initiate the reaction, the assay was carried out at 25 °C using an Envision 2140 multilabel reader (PerkinElmer, Waltham, MA) fitted with thermostatically controlled