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Stimulation of the brain serotonin receptor 7 rescues mitochondrial dysfunction in female mice from two models of Rett syndrome

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ABSTRACT

Rett syndrome (RTT) is a rare neurodevelopmental disorder, characterized by severe behavioral and physiological symptoms. Mutations in the methyl CpG binding protein 2 gene (*MECP2*) cause more than 95% of classic cases, and currently there is no cure for this devastating disorder. Recently we have demonstrated that neurobehavioral and brain molecular alterations can be rescued in a RTT mouse model, by pharmacological stimulation of the brain serotonin receptor 7 (5-HT7R). This member of the serotonin receptor family, crucially involved in the regulation of brain structural plasticity and cognitive processes, can be stimulated by systemic repeated treatment with LP-211, a brain-penetrant selective agonist. The present study extends previous findings by demonstrating that LP-211 treatment (0.25 mg/kg, once per day for 7 days) rescues mitochondrial respiratory chain impairment, oxidative phosphorylation deficiency and the reduced energy status in the brain of heterozygous female mice from two highly validated mouse models of RTT (MeCP2-308 and MeCP2-Bird mice). Moreover, LP-211 treatment completely restored the radical species overproduction by brain mitochondria in the MeCP2-308 model and partially recovered the oxidative imbalance in the more severely affected MeCP2-Bird model. These results provide the first evidence that RTT brain mitochondrial dysfunction can be rescued targeting the brain 5-HT7R and add compelling preclinical evidence of the potential therapeutic value of LP-211 as a pharmacological approach for this devastating neurodevelopmental disorder.

1. Introduction

Rett syndrome (RTT) is a rare neurodevelopmental disorder, characterized by severe behavioral and physiological symptoms (Hagberg, 2002; Rett, 1966). One essential feature of RTT is the apparently normal perinatal development until about 6–18 months of age, when RTT patients start losing their acquired cognitive, social, and motor skills and develop a wide variety of symptoms, including autistic-like behaviors, anxiety, motor disturbances, stereotypic hand movements and severe cognitive dysfunction (Hagberg, 2002). Mutations in the methyl CpG binding protein 2 gene (*MECP2*) cause more than 95% of classic cases (Amir et al., 1999; Chahrour and Zoghbi, 2007; Guy et al., 2001). *MeCP2* encodes a multifunctional protein that binds to methylated DNA and mainly acts as a key transcriptional regulator (Guy et al., 2011). How mutations in the *MeCP2* gene lead to the neurobehavioral features of RTT is still unknown and there is no cure for this devastating disorder.

We recently demonstrated that stimulation of central serotonin receptor 7 (5-HT7R) with LP-211, a brain penetrant selective agonist which binds with high affinity at the human cloned 5-HT7R

Abbreviations: 5-HT7R, 5-hydroxytryptamine, serotonin receptor 7; ASC, ascorbate; BSA, bovine serum albumin; H_2O_2, hydrogen peroxide; HOVA, homovanillic acid; GLU, glutamate; MAL, malate; MeCP2, methyl CpG binding protein 2 gene; MRC, mitochondrial respiratory chain; OXPHOS, oxidative phosphorylation; SOD, superoxide dismutase; SUCC, succinate; TMPD, *N,N,N′,N′*-tetrametil-*p*-fenilendiammina; ROS, reactive oxygen species; RTT, Rett syndrome; wt, wild-type.

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(Hedlund et al., 2010; Leopoldo et al., 2008, 2011), substantially rescues the neurobehavioral phenotype in a mouse model of RTT (De Filippis et al., 2014a, 2015a). 5-HT7R is the most recently discovered serotonin receptor and is involved in a number of neuro-physiological phenomena relevant for RTT, including regulation of the circadian rhythm, sleep, mood and cognitive processes, and in the regulation of structural plasticity in brain circuits (Canese et al., 2014; Gasbarri and Pompili, 2014; Meneses, 2014; Volpicelli et al., 2014). Consistent with these observations, 5-HT7R activation stimulates signaling cascades known to play a prominent role in synaptic plasticity and cognition, such as the extracellular-signal regulated kinases (ERKs), the cyclic AMP protein kinase (PKA) and the Cyclin-dependent kinase 5 (Cdk5) (Guseva et al., 2014; Volpicelli et al., 2014).

Notably, stimulation of the central 5-HT7R with LP211 can also activate in mouse brain the Rho GTPases, which are low-molecular-weight guanine nucleotide binding proteins critically involved in different forms of intellectual disabilities (De Filippis et al., 2014b; Etienne-Manneville and Hall, 2002), and can rescue the abnormal activation of Rho GTPases effectors in RTT mouse brain (De Filippis et al., 2012). This family of proteins regulates a variety of important processes, including vesicle transport, microtubule dynamics, cell-cycle progression and gene expression (Feltri et al., 2008; Hall, 2005; Luo, 2000; Nakayama et al., 2000; Tashiro et al., 2000). From a molecular point of view, Rho GTPases have been historically linked to signaling pathways related to cytoskeletal remodeling (Ramakers, 2002); aberrant Rho GTPases signaling is in fact critically associated to cognitive dysfunction and to the accompanying alterations in dendritic spine morphology (Bassani et al., 2013; Ramakers, 2002). Recent evidence also suggests a role for Rho GTPases in the regulation of a signaling pathway critically involved in the regulation of local protein synthesis (De Filippis et al., 2014b; De Rubeis et al., 2013).

Recently, we have provided innovative evidence that Rho GTPases may be critically involved in the regulation of brain mitochondria (De Filippis et al., 2015b, 2015c), whose dysfunction has been indicated as a central player in several pathological conditions associated with intellectual disabilities (Valenti et al., 2014a). This was achieved by intracerebroventricular (icv) administration in mouse brain of CNF1, a bacterial protein produced by several strains of *Escherichia coli* (De Filippis et al., 2012; Loizzo et al., 2013). The CNF1 bacterial protein specifically activates the Rho GTPases through its C-terminal catalytic domain (Fabbri et al., 2013). We demonstrated that specific activation of Rho GTPases by CNF1 in a RTT mouse model improves the neurobehavioral phenotype and rescues in the brain the defective oxidative phosphorylation (OXPHOS) apparatus, the mitochondrial molecular machinery responsible for the majority of cell energy production. The mitochondrial overproduction of H_2O_2 associated with the decrease in brain energy status was also contrasted by CNF1 in RTT mouse brain (De Filippis et al., 2015b, 2015c). Importantly, the use as a control treatment of a mutant CNF1 protein whose Rho enzymatic activity was abrogated (Fabbri et al., 2013), allowed us to unequivocally confirm the pivotal role of Rho GTPases in the rescue of mitochondrial dysfunction in CNF1-treated RTT mouse brains. This also provided compelling evidence that modulation of brain Rho GTPases affects brain mitochondrial functionality.

Based on this evidence, we argued that modulation of Rho GTPases signaling by 5-HT7R stimulation might have similar beneficial effects on brain mitochondrial defects for RTT. This has important implication from a translational point of view, because the LP-211 treatment can be systematically administered, thus bearing a higher clinical relevance compared to the CNF1 icv administration.

The present study thus verified whether repeated systemic treatment with LP-211 rescues mitochondrial dysfunction and the subsequent redox imbalance in RTT mouse brain. To this aim, we applied the same LP-211 treatment schedule we have previously reported to exert long-term beneficial effects on behavioral and molecular alterations in a mouse model of RTT (De Filippis et al., 2015a). To substantiate our results, the study was carried out in symptomatic heterozygous female mice, the genetic and hormonal milieus that more closely resemble those of RTT patients. To strengthen our results, the study was carried out using two highly validated mouse models of RTT: (i) the MeCP2-308 model, that bears a truncating mutation, leading to the expression of a truncated protein (De Filippis et al., 2010; Shahbazian et al., 2002); (ii) the MeCP2-Bird model that bears a null mutation (Guy et al., 2011). In agreement with clinical data from RTT patients carrying C-terminal deletions of the MeCP2 gene (Díaz de León-Guerrero et al., 2011), MeCP2-308 hemizygous male mice present a delayed onset of symptoms and a prolonged life-span in comparison with knockout male mice from the MeCP2-Bird model (Ricceri et al., 2008, 2013).

2. Materials and methods

2.1. Subjects

The experimental subjects were 8–10 months old heterozygous female mice and wild-type (wt) littermates from two strains: the MeCP2-308 strain [B6.129S-MeCP2tm1Heto/J, stock number: 005439] or the MeCP2-Bird strain [B6.129P2(C)-Mecp2 tm1.1Bird/J, Stock No: 003890] from the Jackson Laboratories (USA), backcrossed to C57BL/6J mice for at least 12 generations.

Mice were housed in groups of 2–3 in polycarbonate transparent cages (33 × 13 × 14 cm) with sawdust bedding and kept on a 12-h light-dark schedule (lights off at 8:00am). Temperature was maintained at 21 ± 1 °C and relative humidity at 60 ± 10%. Animals were provided *ad libitum* with tap water and a complete pellet diet (Altromin, Germany). All procedures were carried out in accordance with the European Communities Council Directive (2010/63/EU) as well as Italian law, and formally approved by Italian Ministry of Health.

2.2. Drug and treatment

LP-211 was prepared following the same synthetic procedure described in (Leopoldo et al., 2008). The compound was dissolved in a vehicle solution of 1% dimethyl sulfoxide (DMSO) in saline (0.9% NaCl). MeCP2-mutated mice and wt littermate controls were randomly assigned to be daily intra-peritoneally (ip) injected (between 9.00 and 11.00 a.m.) for 7 consecutive days with either LP-211 (0.25 mg/kg) or vehicle (1% of DMSO in saline). The dose was chosen based on previous studies (Adriani et al., 2012; De Filippis et al., 2015a).

2.3. Mitochondrial analysis

One month after the last ip injection of LP-211 or control, MeCP2-mutated heterozygous females and their wt littermates were sacrified and the brains were explanted. The estrous status of the experimental mice at the time of the sacrifice was not controlled, based on recent evidence demonstrating that brain mitochondrial function is not affected by hormonal fluctuations during the estrous cycle (Gaignard et al., 2015).

Immediately after their explantation, the brains were added to an ice-cold cryopreservation solution consisting of 50 mM K-MES (pH 7.1), 3 mM K_2HPO_4, 9.5 mM $MgCl_2$, 3 mM ATP plus 20% glycerol and 10 mg/ml BSA, and stored at −80 °C until assayed. Previous