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Research Paper

Are dopamine receptor and transporter changes in Rett syndrome reflected in Mecp2-deficient mice?

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ABSTRACT

We tested the claim that the dopaminergic dysfunction of Rett Syndrome (RTT) also occurs in Mecp2-deficient mice that serve as a model of the syndrome. We used positron emission tomography (PET) to image dopamine D_2 receptors (D_2R) and transporters (DAT) in women with RTT and in Mecp2-deficient mice, and D_1R and D_2R density was measured in postmortem human tissue by autoradiography. Results showed 1) significantly reduced D_2R density in the striatum of women with RTT compared to control subjects. 2) PET imaging of mouse striatum similarly demonstrated significant reductions in D_2R density of 7–10 week-old hemizygous (*Mecp2*-null) and heterozygous (HET) mice compared to wild type (WT) mice. With age, the density of D_2R declined in WT mice but not HET mice. 3) In contrast, postmortem autoradiography revealed no group differences in the density of D_1R and D_2R in the caudate and putamen of RTT *versus* normal control subjects. 4) In humans and in the mouse model, PET revealed only marginal group differences in DAT. The results confirm that dopaminergic dysfunction in RTT is also present in Mecp2-deficient mice and that reductions in D_2R more likely explain the impaired ambulation and progressive rigidity observed rather than alterations in DAT.

1. Introduction

Rett Syndrome (RTT) is a developmental disorder with cognitive, motor, sensory, emotional, and autonomic functional impairments (Dunn, 2001; Hagberg, 2002; Naidu et al., 1995; Percy et al., 2010), including stereotyped limb movements, dystonia, dyskinesias, progressive rigidity, and profound intellectual disability (Hagberg, 1989; Humphreys and Barrowman, 2016; Kerr, 1995; Percy et al., 2010; Segawa, 2001). > 90% of patients with the RTT phenotype have mutations in the *MECP2* gene (Bebbington et al., 2008; Hoffbuhr et al., 2001; Percy et al., 2007). The present investigation characterized the expression of dopaminergic D_1 and D_2 receptors (D_1R and D_2R), and dopamine transporter (DAT) availability (radioligand binding potentials) in human RTT and in Mecp2-deficient mice. By relating findings in humans to consequences of the gene mutation in mice, we tested the claim that Mecp2-deficient mice qualify as a model of the dopaminergic deficits in human RTT.

Early studies of girls with RTT demonstrated reductions in levels of dopamine (DA) and its metabolite homovanillic acid (HVA) in plasma (Riederer et al., 1985), cerebral spinal fluid (CSF) (Percy et al., 1985; Zoghbi et al., 1989; Zoghbi et al., 1985) and in postmortem brain tissue of patients with RTT (Lekman et al., 1989; Riederer et al., 1986; Wenk, 1995; Wenk et al., 1991), while other studies failed to reveal changes in CSF HVA level (Lekman et al., 1990; Perry et al., 1988). In RTT, levels of tyrosine hydroxylase, the rate limiting enzyme for DA synthesis, also were decreased in the substantia nigra pars compacta (SNpc), the origin

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of the nigrostriatal pathway (Jellinger et al., 1988; Lekman et al., 1989). Postmortem tissue from girls with RTT showed reduced levels of DA in putamen and cerebral cortex and of choline acetyltransferase in caudate nucleus (Wenk et al., 1991), suggesting significant perturbation of nigrostriatal and basal forebrain pathway activities. While reductions in extracellular DA concentrations normally lead to up-regulation of DA receptors, postmortem brains of three patients with RTT had reduced [^{3}H]spiroperidol binding to the D_2R compared to control brains (Riederer et al., 1986). The reductions in DA receptors occurred in the context of reductions in the volume of the caudate nuclei and mid-brain regions (Reiss et al., 1993), suggestive of atrophy that can bias determinations of dopamine receptor density.

Mecp2-deficient mice have enabled the examination of possible specific effects of Mecp2 deficiency on neurotransmitter levels. Mecp2-deficient mice have impaired DA function (Gantz et al., 2011; Panayotis et al., 2011a; Panayotis et al., 2011b) and decreased biogenic amine concentrations (Ide et al., 2005; Panayotis et al., 2011a; Viemari et al., 2005), suggesting that the Mecp2 deficiency has significant effects on several neurotransmitter systems, as also seen in patients with RTT.

We compared the densities of D_2R and DAT *in vivo* in women with RTT (*versus* healthy control subjects) and Mecp2-deficient mice (*versus* wildtype (WT) mice) to determine 1) whether there was dopaminergic pre- and post-synaptic dysfunction in MeCP2 deficient human and mouse brains, and 2) whether the mouse brain exhibits the same brain pathology observed in humans. We measured D_2R density by mapping D_2-like DA receptors *in vivo* in humans and mice (Wong et al., 1986a; Wong et al., 1986b; Wong et al., 1997a; Wong et al., 1997b) and by receptor autoradiography in postmortem human striatum. We also measured dopamine transporter (DAT) density as an index of the number of nigrostriatal terminals *in vivo* in humans and mice. Together these measures validate the use of mice with Mecp2 deficiency as models for the integrity of dopaminergic neurotransmission in women with RTT.

2. Materials and methods

Patients with RTT and healthy volunteers: We included women diagnosed with RTT, based on clinical criteria and mutations in *MECP2*, and normal age-matched women volunteers. Consent was given by the appropriate family members or legal guardians, or by the women themselves, following the rules of the Johns Hopkins University Investigational Review Board. Table 1 lists the age, PET scanner, *MECP2* mutation status, and primary neurological manifestations for the women with RTT studied. The age of the subjects studied ranged from 15 to 32 years of age.

2.1. Mice

The Animal Care and Use Program at Johns Hopkins University approved our mouse study protocol. Genotyping of mice was performed using the DNAeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA), as in our previous publications (Blue et al., 2015; Metcalf et al., 2006). For D_2R PET studies, we included 15 *Mecp2*-heterozygous (HET) and 6 *Mecp2*-null mice (Adrian Bird model (Guy et al., 2001)) that ranged in age from 7 to 33 weeks of age. For DAT studies, we included 11 WT, 5 *Mecp2*-null mice and 6 HET mice that were 7–17 weeks of age. For volumetric studies, we included mice at 4 (6 WT, 7 HET, 8 *Mecp2*-null), 7 (7 WT, 6 HET, 8 *Mecp2*-null) and 14 weeks (8 WT, 7 HET, 6 *Mecp2*-null) of age.

2.2. Positron emission tomography (PET)

Magnetic resonance imaging (MRI) (for humans) and computerized tomography (CT) (for mice) were obtained for purposes of localization and anatomic correlation with subsequent PET imaging studies. PET studies for D_2R included two cohorts of patients. The first cohort included 6 women with RTT and 9 controls who had tomography in the CTI NeuroEcat scanner (Siemens CTI, Knoxville, TN, USA). The second cohort of 4 women with RTT and 7 controls later had higher resolution tomography with the GE4096+ whole body PET device that allowed the caudate and putamen to be distinguished. Each participant (healthy or RTT) received 2 PET sessions (baseline and blocked conditions) using 3-*N*-[^{11}C]methylspiperone ([^{11}C]NMSP) at high specific activity, namely, 74-111GBq/μmole (2–3 Ci/μmole) (Dannals et al., 1986) in order to calculate absolute D_2R B_{max}. For the CTI NeuroECAT studies, subjects had up to 12 acquisitions during the 90-min scans (Wong et al., 1986a; Wong et al., 1986b; Wong et al., 1997a).

To increase the number of subjects in each group, we joined the results of each study after normalization of data obtained with the different tomographs together. For the second cohort we normalized the values by averaging the values for the caudate and putamen. We then calculated the mean value for each control group in each cohort and expressed the values for each subject as percent control and performed statistical analysis for the two cohorts together.

Table 1
Clinical data for human patients in PET imaging study. Clinical data about females with Rett syndrome (RTT) who underwent PET scans to estimate the density of D_2 dopamine receptors (D_2R) and of dopamine transporters (DAT) in the striatum. Two women indicated by (*) and ($^+$) underwent both procedures. Abbreviations: Rigid = rigidity; Trem = tremor; val = valproate; carb = carbamazepine; top = topiramate; phen = phenobarbital.

Study	Age	PET Scanner	*MECP2* Mutation	Seizures	Walking	Rigid	Trem	Scoliosis	Medicines
D2	15	GE 4096	P225R	−	+	+	+	+	none
D2	15	GE 4096	R306C	+	−	+	−	−	none
D2	21	GE 4096	1155 (55 DEL)	+	+	+	+	+	none
D2	32	GE 4096	R306C	−	+	+	+	+	val
D2	15	NEUROECAT	T158 M	+	+	+	−	+	none
D2*	19	NEUROECAT	R168X	+	+	+	+	+	carb
D2	19	NEUROECAT	R106W	+	+	+	+	+	none
D2	21	NEUROECAT	R270X	+	+	+	+	+	none
D2	24	NEUROECAT	R270X	+	+	+	+	+	none
D2$^+$	26	NEUROECAT	R133C	+	−	+	−	+	carb
DAT	18	GE 4096	T158 M	−	+	+	+	+	none
DAT	18	GE 4096	R270X	+	+	+	+	−	carb
DAT	19	GE 4096	R106W	+	−	+	+	+	top,phen
DAT*	20	GE 4096	R168X	+	+	+	+	+	carb
DAT	20	GE 4096	R294X	−	+	+	+	+	none
DAT	21	GE 4096	T158 M	+	+	+	+	+	val
DAT	21	GE 4096	T158 M	−	+	+	+	+	none
DAT	24	GE 4096	R294X	−	+	+	+	+	none
DAT$^+$	26	GE 4096	R133C	+	−	+	−	+	carb