Priority Communication

Biological Psychiatry

An Early Postnatal Oxytocin Treatment Prevents Social and Learning Deficits in Adult Mice Deficient for *Magel2*, a Gene Involved in Prader-Willi Syndrome and Autism

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ABSTRACT

BACKGROUND: Mutations of *MAGEL2* have been reported in patients presenting with autism, and loss of *MAGEL2* is also associated with Prader-Willi syndrome, a neurodevelopmental genetic disorder. This study aimed to determine the behavioral phenotype of *Magel2*-deficient adult mice, to characterize the central oxytocin (OT) system of these mutant mice, and to test the curative effect of a peripheral OT treatment just after birth.

METHODS: We assessed the social and cognitive behavior of *Magel2*-deficient mice, analyzed the OT system of mutant mice treated or not by a postnatal administration of OT, and determined the effect of this treatment on the brain.

RESULTS: *Magel2* inactivation induces a deficit in social recognition and social interaction and a reduced learning ability in adult male mice. In these mice, we reveal anatomical and functional modifications of the OT system and show that these defects change from birth to adulthood. Daily administration of OT in the first postnatal week was sufficient to prevent deficits in social behavior and learning abilities in adult mutant male mice. We show that this OT treatment partly restores a normal OT system. Thus, we report that an alteration of the OT system around birth has long-term consequences on behavior and on cognition. Importantly, an acute OT treatment of *Magel2*-deficient pups has a curative effect.

CONCLUSIONS: Our study reveals that OT plays a crucial role in setting social behaviors during a period just after birth. An early OT treatment in this critical period could be a novel therapeutic approach for the treatment of neurodevelopmental disorders such as Prader-Willi syndrome and autism.

Keywords: Autism spectrum disorder, MAGEL2, Neurodevelopment, Oxytocin, Prader-Willi syndrome, Therapy

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Oxytocin (OT) produced in the central nervous system is released directly into the brain (hereinafter named central OT) and into the blood circulation via the pituitary gland. Recently, the role of central OT has become increasingly reported. In rodents, central OT is produced by magnocellular and parvocellular hypothalamic neurons mainly located in the paraventricular (PVN) and supraoptic nuclei. These neurons projecting to various central brain areas act as neuromodulators to regulate several behavioral functions (1–6). A significant body of data points to OT playing a role in both childhood and adult neuropsychiatric disorders, characterized by social and cognition impairments (7–9). Numerous clinical trials in adult patients, using OT administration and targeting a diversity of psychiatric disorders, including autism spectrum disorder (ASD) (10), are currently under way. However, in patients, these OT treatments are empiric, and in animal models, they have given controversial results raising questions about the validity of this therapeutic approach. The strength of the scientific rationale should be seriously considered to define an optimized therapy (11). Considering the substantial heterogeneity of human disorders with social and cognition impairment and the lack of appropriate mouse models, this question is particularly challenging.

Prader-Willi syndrome (PWS) is one of the best studied neurodevelopmental diseases, characterized mainly as a feeding disorder with behavioral and social disturbances (12–16). At birth, 80% of newborns have poor suckling activity requiring tube feeding (15,17,18). After 2 years, children develop a true hyperphagia leading to severe obesity (19). Patients present mild to moderate intellectual disability (20) and behavioral problems, including repetitive and ritualistic behaviors and difficulty with routine changes (16,21,22). They have great emotional lability and a deficit in understanding social codes and the environment (23,24). All PWS patients share features of ASD (23) and some atypical PWS patients are diagnosed as autists (25). Although no comprehensive pathophysiological mechanisms have been clearly identified, much of the PWS phenotype is consistent with a hypothalamic dysfunction. Furthermore, the number of OT-expressing neurons is modified in brains of postmortem patients (26).

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MAGEL2 (27,28) is a candidate gene for PWS. Pathogenic mutations of *MAGEL2* gene have been recently reported in patients presenting ASD, a developmental delay/intellectual disability, and feeding problems during infancy (25). These results underline the contribution of *MAGEL2* to cognitive and behavioral alterations in autism and in PWS.

In mouse, *Magel2* is highly expressed in postmitotic hypothalamic neurons from early embryogenesis, and it presents a circadian pattern into adulthood (29,30). We created a mouse model deficient for *Magel2* (*Magel2*$^{tm1.1Mus}$) that showed an altered onset of suckling activity and subsequent impaired feeding, leading to 50% neonatal lethality and affecting both male and female mice (31). *Magel2*-deficient neonates had a reduced quantity of OT and a single shot of OT was sufficient to immediately restore a normal suckling activity, allowing all pups to survive (31).

In the present study, we analyzed the effects of *Magel2* inactivation in the 50% surviving adult mice, and we showed deficiencies in learning abilities and social behavior, affecting male mice only. Early postnatal OT treatment was used as a long-term therapeutic approach to prevent behavioral and learning deficits in adulthood. We also investigated alterations of the OT system (OT neurons, OT-targeted brain structures, and OT receptors) in adult mutant male mice and the effects of an early OT treatment on these alterations. Finally, we demonstrated that OT administered in the periphery reaches the brain within minutes and might stimulate central OT release.

METHODS AND MATERIALS

Breeding and Mice Cohorts

Mice were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (32) and the European Communities Council Directive of September 22th 2010 (2010/63/EU, 74). Experimental protocols were approved by the institutional ethical committee guidelines for animal research (accreditation no. B13-055-19 from the French Ministry of Agriculture). *Magel2*-deficient mice were generated as previously described (31). Mice colonies were maintained on the C57BL/6J background. *Magel2* was submitted to genomic imprinting, with expression of the inherited paternal allele only. We thus transmitted the paternal mutation in the generating litters crossing heterozygote males −m/+p with wild-type (WT) C57BL/6J female mice to get half offspring WT (+/+) as control mice and half deficient for the *Magel2* paternal allele (*Magel2*$^{+m/-p}$). Considering the circadian expression of *Magel2*, we killed all the adult animals in the same window of time in all experiments. Three cohorts of mice described in Supplement 1 were used.

Behavioral Tests

The effects of *Magel2*$^{+m/-p}$ mutation were evaluated on the general heath and motor abilities, different aspects of learning and memory processes, and social behaviors (Supplement 1).

OT Treatment

Three to 5 hours after delivery, WT or *Magel2*$^{+m/-p}$ pups were given a single subcutaneous injection (20 μL) of isotonic saline or oxytocin (2 μg) (Phoenix Pharmaceuticals. Inc., Strasbourg, France; Catalog No.051-01) dissolved in isotonic saline (20 μL). For a daily OT injection in the first week of life, a single subcutaneous injection of oxytocin was performed 3 to 5 hours after birth, then identical injections were performed daily for the 6 following days.

Antibodies, Immunofluorescence, Microscopy, and Quantitative Analysis

Brain cryosections (20 μm) were collected from the anterior commissure to the posterior hypothalamus from 4% Antigen-Fix (Diapath, Martineng, Italy) perfused and frozen brains. We used a mouse monoclonal antibody directed against a nonspecific form of OT (MAB5296,1/250; Chemicon, Merck Millipore, Darmstadt, Germany). We also performed co-immunolabeling using the PS38 mouse monoclonal antibody (1/50) highly specific against the mouse neurophysin associated to OT (33) and the VA10 polyclonal antibody (1/1000) that recognizes the intermediate forms of OT (34). PS38 and VA10 were gifts from Dr. H. Gainer, PhD, NINDS, National Institutes of Health, Bethesda, Maryland. Quantitative analysis is described in Supplement 1.

Autoradiography of OT Binding Sites

Localization of oxytocin binding sites was performed according to a previously published protocol [(35) and Supplement 1].

Quantification of OT in Adult Hypophysis and Hypothalamus

Dosages were performed on adult animals of the same age (40 weeks old), killed in the same window of time (12–14 pm) by beheading, and followed by dissection of hypothalamus and hypophysis.

Enzyme-linked immunoassay analyses to quantify OT and arginine vasopressin (AVP) were performed as described in Supplement 1.

Mass spectrometry analyses and matrix-assisted laser desorption/ionization (MALDI) are described in Supplement 1.

Statistical Analysis

Concerning behavioral studies, data were analyzed using unpaired Student *t* test or repeated measures analysis of variance with one or two between factors (genotype, sex) and one within factor (time, quadrant). Qualitative parameters (e.g., clinical observations) were analyzed using the chi-square test. Concerning anatomical parameters, nonparametric statistical software (StatXact; Cytel Software Corporation, Cambridge, Massachusetts) were used. All tests are two-tailed tests. In the results, values are indicated as: Q2 (Q1, Q3), *n*, *p* value, where Q2 is the median, Q1 is the first quartile, and Q3 is the second quartile. The level of significance was set at a p value $< .05$.

RESULTS

Alterations in Social Behavior and Learning and Memory Abilities in Adult *Magel2*$^{+m/-p}$ Male Mice

The effects of the *Magel2* mutation were assessed on the surviving *Magel2*$^{tm1.1Mus}$ mice, those with a deletion on the