Neuroscience Letters 589 (2015) 57–61

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Reduced susceptibility to induced seizures in the Neuroligin-3^{R451C} mouse model of autism

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HIGHLIGHTS

- Seizure susceptibility was examined in the Neuroligin-3^{R451C} mouse model of autism.
- Reduced susceptibility to tonic–clonic seizures in Neuroligin-3^{R451C} mice.
- Regional specific changes in the ratio of excitatory/inhibitory neurotransmission may influence seizure susceptibility to specific stimuli.

ARTICLE INFO

Article history:
Received 18 December 2014
Accepted 9 January 2015
Available online 12 January 2015

Keywords:
Autism
Mice
Epilepsy
Seizures
Tonic–clonic
Pentylenetetrazole

ABSTRACT

Epilepsy is a common comorbidity in patients with autism spectrum disorder (ASD) and several gene mutations are associated with both of these disorders. In order to determine whether a point mutation in the gene for the synaptic protein, Neuroligin-3 (Nlgn3, R451C), identified in patients with ASD alters seizure susceptibility, we administered the proconvulsant pentylenetetrazole (PTZ) to adult male Neuroligin-3^{R451C} (NL3^{R451C}) and wild type (WT) mice. It has previously been reported that NL3^{R451C} mice show altered inhibitory GABAergic activity in brain regions relevant to epilepsy, including the hippocampus and somatosensory cortex. PTZ administration induces absence-seizures at low dose, and generalised convulsive seizures at higher dose. Susceptibility to absence seizures was examined by analysing the frequency and duration of spike-and-wave discharge (SWD) events and accompanying motor seizure activity induced by subcutaneous administration of low dosage (20 or 30 mg/kg) PTZ. Susceptibility to generalised convulsive seizures was tested by measuring the response to high dosage (60 mg/kg) PTZ using a modified Racine scale. There was no change in the number of SWD events exhibited by NL3^{R451C} compared to WT mice following administration of both 20 mg/kg PTZ (1.17 ± 0.31 compared to 16.0 ± 11.16 events/30 min, NL3^{R451C} versus WT, respectively) and 30 mg/kg PTZ (7.5 ± 6.54 compared with 27.8 ± 19.9 events/30 min, NL3^{R451C} versus WT, respectively). NL3^{R451C} mice were seizure resistant to generalised convulsive seizures induced by high dose PTZ compared to WT littermates (median latency to first >3 s duration clonic seizure; 14.5 min versus 7.25 min, 95% CI: 1.625–2.375, $p = 0.0009$, NL3^{R451C} versus WT, respectively). These results indicate that the R451C mutation in the *Nlgn3* gene, associated with ASD in humans, confers resistance to induced seizures, suggesting dysfunction of PTZ-sensitive GABAergic signalling in this mouse model of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a heterogenous disorder in which patients demonstrate impairments in social communication alongside repetitive behaviors and/or restricted interests. Many (40–80%) patients with autism spectrum disorder (ASD) also have epilepsy as a comorbid condition [7,24,31] and approximately 60%

Abbreviations: ECoG, electrocorticogram; NL3, Neuroligin-3; PTZ, pentylenetetrazole; SWD, spike-and-wave discharge; ASD, autism spectrum disorder.

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http://dx.doi.org/10.1016/j.neulet.2015.01.024
0304-3940/

of ASD patients without a history of epilepsy show abnormal EEG activity during sleep [6]. Infantile spasms are an independent risk factor for some forms of syndromic autism (i.e., tuberous sclerosis complex) [16]. Several genetic mutations (many encoding synaptic proteins) are associated with both epilepsy and autism in patients [1,4,11,25] and common pathophysiological underpinnings have been proposed. For example, changes in neuronal connectivity and synchrony [36], alterations in synaptic plasticity and abnormal ratios of cortical excitatory/inhibitory neurotransmission [2,30] have been implicated in ASD and epilepsy.

Several mutations in genes involved in synaptic function have been associated with ASD in patients (reviewed in Betancur, 2009 [5]). The R451C missense mutation in the *Nlgn3* gene encoding the Neuroligin-3 (NL3) protein was identified in two siblings with ASD, one with comorbid epilepsy [21]. Transgenic mice carrying this mutation ($NL3^{R451C}$ mice) exhibit a number of phenotypes relevant to ASD in humans [14,29,33]). Seizure susceptibility has not been investigated in $NL3^{R451C}$ mice. However, Radyushkin et al., [28] studied NL3 null mice bred on a C57/Bl6 background strain and found no change in seizure susceptibility following subcutaneous administration of the proconvulsive drug, pentylenetetrazole (PTZ; 50 mg/kg).

$NL3^{R451C}$ mice show regional-specific changes in GABAergic and glutamatergic neurotransmission in brain slices [13,14,27,33]. $NL3^{R451C}$ mutants exhibit enhanced excitatory input in the hippocampus together with increased inhibitory neuronal activity in the somatosensory cortex [13,27,33]. In addition, endocannabinoid-mediated inhibitory activity is altered in hippocampal neurons of both $NL3^{R451C}$ and NL3 knockout mice, suggesting that Neuroligin-3 is essential for tonic endocannabinoid signalling [14]. Both $NL3^{R451C}$ and NL3 knockout mice showed impaired synaptic inhibition onto D1-dopamine receptor expressing medium spiny neurons in ventral striatum and heightened repetitive behaviors [29], further suggesting that an imbalance in inhibitory neurotransmission contributes to the phenotype of these mice.

Administration of PTZ is routinely used to test seizure susceptibility (SWD events and tonic–clonic seizures) in animal models. Low doses (e.g., 20–30 mg/kg) of PTZ induce an absence seizure phenotype in rodents with episodes of generalised spike-and-wave discharge (SWD) on the EEG associated with behavioral arrest [22,32]. Higher doses (i.e., 50 mg/kg-85 mg/kg) induce convulsive seizures with generalised tonic–clonic motor activity [9,19]. In this study we tested whether $NL3^{R451C}$ mice exhibit altered susceptibility to absence and convulsive seizures induced by PTZ in order to investigate whether this mutation could play a mechanistic role in the co-morbidity of ASD and epilepsy.

2.1. Methods animals

B6;129-$Nlgn3^{tm1Sud/J}$ mice were obtained from Jackson Laboratories (Bar Harbor, Maine USA) and maintained to generation F9 on a hybrid Sv129/C57Bl6 background. $NL3^{R451C}$ and WT animals were derived by mating heterozygous females with $NL3^{R451C}$ males, which produced 50:50 WT and $NL3^{R451C}$ male offspring (Y/+ and Y/R451C) and genotyped as described [33]. Experimental animals (adult male mice aged 12–15 weeks) were weaned at 4 weeks of age and housed in groups of four per cage with food and water available ad libitum. The holding room was maintained on a 12:12 h light/dark cycle with lights on at 7 a.m. and at an ambient temperature of $20 \pm 1\,^{\circ}C$. All procedures were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee.

2.2. Surgery

Prior to the surgical procedure, mice were administered 1 ml of 0.9% saline and 1 ml/kg of the anti-inflammatory caprofen (1:100 dilution, Sigma–Aldrich, Australia) in sterile water and administered via subcutaneous injection. Mice were anaesthetized by inhalation of isoflurane (Abbott Pharmaceuticals, USA) in equal parts of medical air and oxygen (5% induction, 1.5–2.5% maintenance during the procedure). Mice were positioned using a stereotaxic frame (WPI 502600, World Precision Instruments, USA) as described previously [15,17,26]. The fur overlying the scalp was shaved and the skin was disinfected using a surgical iodine solution (Povidone Iodine, Lyppard Pty Ltd). A midline incision (2 cm in length) was made over the scalp and tissue overlying the cranium was cleared with 0.1% hydrogen peroxide solution in distilled water. The stereotaxic frame was used to measure and mark the skull at coordinates relative to bregma. Six holes were drilled into the skull: 2 mm anterior and 2 mm lateral to bregma bilaterally (active electrodes); 2 mm posterior and 2 mm lateral to bregma bilaterally (ground electrodes); and 6 mm posterior and 1.5 mm lateral to lambda bilaterally (reference electrodes) using a 0.1 mm diameter diamond coated dental drill (Vertex, Zeist, The Netherlands). Stainless steel anchor screws attached to brass recording electrodes (both 0.6 mm diameter; Plastics One USA) were implanted into the skull without breaching the dura and fixed into place with dental cement (Vertex™ Self-Curing Quick Set Henry Schein Halas Australia). The anterior and posterior excision margins were sutured (Dysilk/3.0, Dynek, Hendon, Australia). Mice were housed individually in cages placed on heatpads (Kent Scientific Corporation, USA) set to a constant temperature of 37 °C for 24 h to assist recovery. Mice were monitored for general health status and weight for one week following surgery.

2.3. Seizure induction

Mice were left in their home cages in the experimental room for at least 30 min to habituate before experiments were commenced. Mice were placed into a clear perspex box (20 cm × 20 cm) and allowed to habituate for 5 min to the environment. PTZ was administered at 20, 30 or 60 mg/kg via a subcutaneous (s.c.) injection (caudal to the cervical vertebrae). Assessment of seizure parameters (SWDs and/or behavioral indications) was conducted during 30 min following PTZ administration. Doses were not randomized due to the fact that 60 mg/kg PTZ often causes death in mice. Some animals tested with low dose PTZ (20 mg/kg; WT = 4, $NL3^{R451C}$ = 5) were also used for higher dose experiments with a minimum period of 24 h between experiments to enable drug washout.

2.4. ECoG data acquisition and processing

To assess for the presence of spike-and-wave discharges (SWDs) following PTZ (20 and 30 mg/kg) administration, ECoG data were recorded via implanted electrodes. Data were acquired using a MacLab amplifier and A–D converter and Chart v.3.5 software (AD Instruments, Australia) at a sampling frequency of 256 Hz. Files were reviewed offline and SWDs identified and quantified using pClampv10.0 software (Axon Instruments, Foster City, CA, U.S.A). The primary endpoints for analysis were: (1) number of SWD events and (2) spiking activity.

2.5. Behavioral analysis of seizure activity

PTZ (60 mg/kg) was administered via s.c injection and motor seizures were scored over a 30 min period by an observer blinded to genotype. Seizure scores were determined by a modified Racine scale for PTZ-induced seizures [23]. Briefly, a score of 0 indicated no