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Down-regulation of RalBP1 expression reduces seizure threshold and synaptic inhibition in mice

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ABSTRACT

Idiopathic epilepsy is characterized by seizures without a clear etiology and is believed to have a strong genetic component but exhibits a complex inheritance pattern. Genetic factors seem to confer a low seizure threshold to susceptible individuals and thereby enhance epileptogenesis. However, the identity of susceptibility genes and the mechanisms regulating seizure threshold are still poorly understood. Here, we describe that reduced expression of RalBP1, a downstream effector of the small GTPases RalA and RalB, lowers the seizure threshold in mice. The intraperitoneal injection of the chemoconvulsant pentylenetetrazol induced more severe seizures in RalBP1 hypomorphic mice than in their wild-type littermates. The reduction of RalBP1 in the brain has no effect on neuronal excitability, but does decrease the inhibitory synaptic transmission onto CA1 pyramidal neurons. This impaired synaptic inhibition was associated with the loss of GABAergic interneurons in the CA1 subfield of the hippocampus. The present study identifies RalBP1 as a gene regulating the seizure threshold in mice and provides direct evidence for the role of RalBP1 in synaptic inhibition in vivo.

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1. Introduction

Epilepsy is a common, highly heritable neurological disorder that is characterized by recurrent and unprovoked seizures [1]. Genetic studies have identified some of the genes responsible for monogenic epilepsy; these genes predominantly encode ion channels or their accessory subunits [2]. These genes have provided opportunities for understanding the mechanisms that cause neuronal hyperexcitability or an imbalance in excitatory and inhibitory synaptic transmission, which are major causes of monogenic epilepsy. However, the genetic etiology of the majority of epilepsy is still unknown because most common epilepsies do not follow a Mendelian pattern of inheritance. Monogenic inheritance accounts for only a few unusual types of epilepsy that exhibit a prevalence of ~1%. In contrast, common epilepsies – idiopathic epilepsies – occur without a clear cause and exhibit complex inheritance patterns [3,4]. In addition, it is well known that environmental factors also influence the onset of common epilepsies [5]. According to this multifactorial threshold model of inheritance, the epileptic phenotype is expressed beyond a critical threshold. The combined action of risk genes and environmental factors determines whether an individual surpasses the critical threshold and develops epileptic disease [6]. In line with this concept, the seizure threshold is partly determined by genetic background. Defects in susceptible genes seem to lower the seizure threshold [7], but the identity of these susceptibility genes and the underlying mechanisms that link genetic background and altered seizure threshold are still poorly understood. These susceptibility genes are of particular interest because they may provide new insights into the mechanism underlying idiopathic epilepsies.

Here, we report that reduced expression of RalBP1 (RLIP76) lowers the seizure threshold in mice. RalBP1 is a downstream effector of the small GTPases RalA and RalB that mediates signaling from Ral GTPases to Rho pathways [8]. RalBP1 contains a Ral binding domain and a RhoGAP domain. The former domain mediates binding to Ral GTPases and the latter domain confers GAP activity to Rac1 and Cdc42. In addition, the N-terminal and C-terminal regions of RalBP1 bind to the AP2 adapter complex and POB1, respectively [9,10]. RalBP1 has been implicated in various cellular processes, including receptor-mediated endocytosis, membrane transport, and mitochondrial fission [11–13]. In neurons, RalBP1 is known to mediate long-term synaptic depression through

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interactions with RalA and PSD-95 [14]. However, link between RalBP1 and seizure threshold has not been studied.

In the present study, we identified RalBP1 as a regulator of seizure threshold and investigated the neural mechanisms associated with altered seizure threshold in mice hypomorphic for RalBP1.

2. Materials and methods

2.1. Animals

Heterozygous RalBP1 (RalBP1$^{+/-}$) mice were generated as previously described [14] and backcrossed with C57BL/6J mice for at least 10 generations. All of the experiments were performed with littermates of both genotypes that were derived from heterozygote crosses. All of the animal maintenance and related experiments were performed according to Institutional Animal Care and Use Committee (IACUC) guidelines for the care and use of animals in research and were approved by the IACUC of Seoul National University (SNU-120113-4).

2.2. Drug administration and seizure scoring

Pentylenetetrazole (Sigma–Aldrich, MO, USA) was dissolved in sterile saline at a concentration of 2 mg/mL and administered by intraperitoneal injection (40 mg/kg). Animals were monitored by video recording for 30 min after the injection. The severity of the behavioral seizures was scored according to the modified Racine scale [15]: score 0, no seizure; score 1, head nodding; score 2, clonic jerks, sporadic shaking; score 3, full-body spasms, Straub tail, rearing; score 4, shrieking, jumping, falling; score 5, violent convulsions, death.

2.3. Slice electrophysiology

The hippocampal slice preparations and electrophysiological recordings were performed as described previously [14,16]. Briefly, hippocampal sections (400 μm) from 4 to 5 week old mice were continuously perfused with aerated (95% O_2, 5% CO_2) recording ACSF (in mM: NaCl 125, $NaHCO_3$ 26, KCl 2.5, NaH_2PO_4 1.25, $MgCl_2$ 1.3, $CaCl_2$ 2.5, D-glucose 10), and whole-cell patch clamp recordings were made using a MultiClamp 700B amplifier (Molecular Devices, CA, USA). Membrane potentials were recorded using a pipette (3–4 MΩ) solution containing (in mM) K-gluconate 110, KCl 20, NaCl 8, HEPES 10, Mg-ATP 4, Na-GTP 0.3, EGTA 0.5 (pH 7.25, 290 mOsm), picrotoxin (50 μM) and NBQX (10 μM) in ACSF. For the mIPSC recordings, the K-gluconate and picrotoxin were replaced by equimolar KCl and D-APV, respectively. During recording, the cells were voltage clamped at −60 mV and TTX (1 μM) was added to the ACSF to inhibit action potential-mediated synaptic transmission. All of the chemicals were purchased from Sigma–Aldrich, with the exception of the picrotoxin, D-APV, and NBQX (Tocris Bioscience, Bristol, UK). The data were analyzed using custom macros written in Igor Pro (WaveMetrics).

2.4. Biocytin labeling and morphometric analysis

For the biocytin labeling, biocytin hydrochloride (0.5%) was dissolved in K-gluconate-based pipette solution. After 10 min in the whole-cell configuration, the patch pipette was gently withdrawn from the cell to form an outside-out patch. The slices were then incubated in ACSF for 1 h to allow the biocytin to diffuse into the dendrites and spines. Subsequently, the slices were fixed with 4% (w/v) paraformaldehyde in phosphate buffered saline (PBS) for 24 h. The slices were then rinsed and incubated with Alexa Fluor 555-conjugated streptavidin (Life Technologies, NY, USA) in PBS (1:200). The slices were rinsed and mounted on glass slides, and images were acquired using a confocal laser scanning microscope (LSM510, Zeiss).

2.5. Immunohistochemistry

The mice were anesthetized with diethyl ether and transcardially perfused with 1X PBS followed by 4% paraformaldehyde in PBS. The mouse brains were post-fixed at 4 °C for 48 h and cut into 60 μm coronal sections. The slices were permeabilized with 0.3% (v/v) Triton-X 100 in PBS for 30 min, blocked with 1% BSA for 3 h, and incubated overnight at 4 °C with anti-GAD67 antibodies (Chemicon, MA, USA) before being incubated with FITC-conjugated secondary antibodies for 2 h. The slices were rinsed with PBS three times between each step. Images were acquired with a fluorescence microscope (BX51WI, Olympus) equipped with a cooled charge-coupled device camera (DP70, Olympus) or a confocal laser scanning microscope (LSM510, Zeiss).

2.6. Statistics

The statistical analyses were conducted using IGOR Pro (WaveMetrics, OR, USA) and SPSS (Statistical Package for the Social Sciences, IBM, NY, USA) software. The data are expressed as the mean ± S.E.M.

3. Results

3.1. RalBP1$^{-/-}$ mice show a reduced seizure threshold to convulsant stimulation

To test the association between RalBP1 and epilepsy, we examined spontaneous seizure activity inRalBP1 hypomorphic (RalBP1$^{-/-}$) mice. The level of RalBP1 protein in the RalBP1$^{-/-}$ brains was reduced to ∼18% of the level in wild-type (WT) brains by the insertion of a gene trapping cassette in the RalBP1 gene [14]. This reduced expression of RalBP1 causes modest growth retardation and a reduced body size. As shown in Fig. 1A, the male RalBP1$^{-/-}$ mice weighed ∼20% less than WT littermates at 8 weeks of age (18.40 ± 0.37 g vs. 23.02 ± 0.26 g, n = 6–7 mice, $p < 0.001$, Student's t-test and Mann–Whitney–Wilcoxon test). However, spontaneous seizures and clonic–tonic activity were not detected in RalBP1$^{-/-}$ mice (data not shown). The RalBP1$^{-/-}$ mice appeared healthy and displayed no appreciable differences except for small body size.

We next examined the effect of reduced RalBP1 expression on seizure threshold by measuring convulsant-induced seizure activity in 8-week-old male mice. The intraperitoneal injection of a threshold dose (40 mg/kg) of pentylenetetrazole (PTZ), a chemical convulsant, induced mild clonic seizures in 4 of the 7 WT animals. Myoclonic jerks were observed infrequently in these animals, but the animals did not display tonic seizures. In contrast, the same dose of PTZ evoked clonic seizures in the majority (5 of 6) of the RalBP1$^{-/-}$ littermates (Fig. 1B). In addition, the clonic seizures in these mice often progressed to tonic seizures (3 of 5) with an elevated tail (Straub tail), salivation, running and leaping, and limb extension (Fig. 1C). Quantitative analysis of the seizure scores revealed a significant difference between the genotypes (Fig. 1D). These results indicate that reduced RalBP1 function is not in itself sufficient for seizure induction, but this reduced expression reduces the seizure threshold to convulsant stimulation.

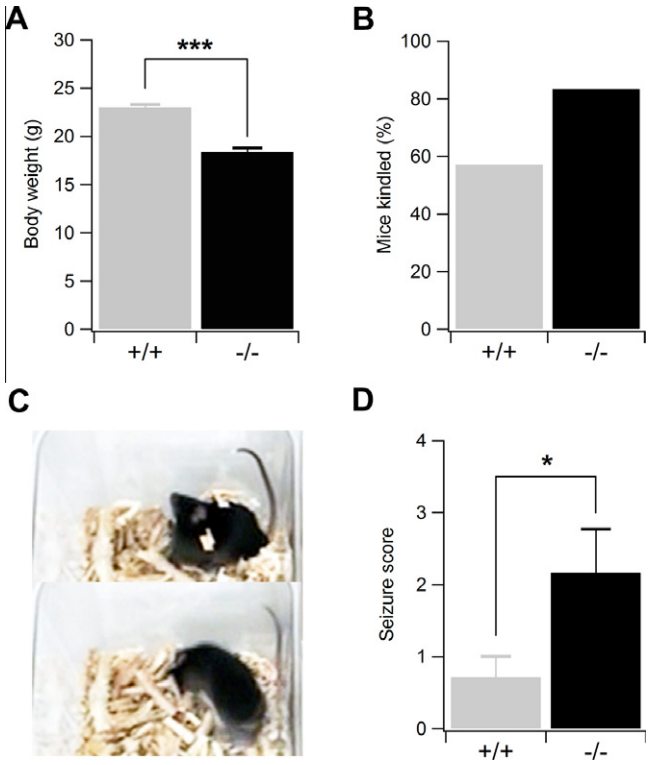

Fig. 1. RalBP1$^{-/-}$ mice are more susceptible to convulsant-induced seizures. (A) The body weight of control WT mice and RalBP1$^{-/-}$ mice 8 weeks after birth. (B) The fraction of WT and RalBP1$^{-/-}$ animals kindled by PTZ-injection. (C) A representative image showing Straub tail (top) and tonic–clonic convulsions (bottom) induced by PTZ injection in RalBP1$^{-/-}$ mice. (D) RalBP1$^{-/-}$ mice show increased sensitivity to PTZ-induced seizures. *$p < 0.05$, Student's *t*-test and Mann–Whitney–Wilcoxon test.

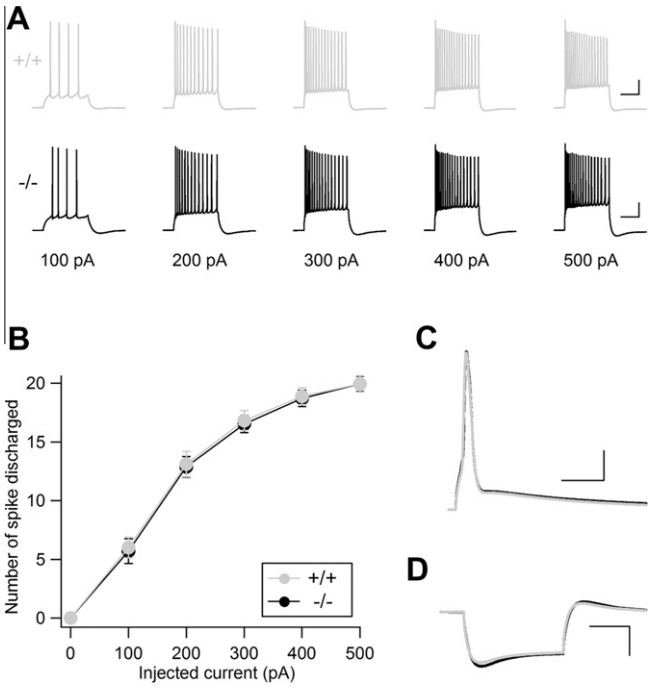

Fig. 2. RalBP1$^{-/-}$ CA1 pyramidal neurons show normal membrane excitability. (A) Representative traces of the spike bursts induced by somatic current injection in WT and RalBP1$^{-/-}$ neurons. The amplitude of the injected current is indicated below the voltage traces. Calibration: 200 ms and 20 mV. (B) Reduced RalBP1 expression had no effect on spike bursts. The number of spikes elicited by a 500 ms current injection was plotted against the amplitude of the injected current. $p > 0.1$, Student's *t*-test. (C) The average action potential traces recorded in WT (gray) and RalBP1$^{-/-}$ neurons (black). Calibration: 10 ms and 20 mV. (D) The average voltage response elicited by a hyperpolarizing current pulse (500 ms, −100 pA) in WT (gray) and RalBP1$^{-/-}$ neurons (black). Calibration: 200 ms and 10 mV. (A–D) $n = 17$–18 neurons from 4 littermates.

3.2. Excitability of RalBP1$^{-/-}$ CA1 neurons

To uncover the mechanism underlying the reduced seizure threshold of RalBP1$^{-/-}$ mice, we analyzed the neural circuits of 4–5 week old mice of both genotypes. Because mouse hippocampal CA1 pyramidal neurons show the strongest expression of RalBP1 protein in the forebrain [14] and the increased excitability of CA1 pyramidal neurons is often associated with enhanced seizure susceptibility [17–19], we first examined the intrinsic neuronal excitability of CA1 neurons. We applied short depolarizing current steps (500 ms, 100 pA) via whole-cell patch pipettes and evoked spike firing. To avoid the erroneous interpretation of spontaneous synaptic inputs, excitatory and inhibitory synaptic transmissions were blocked by NBQX (10 μM) and picrotoxin (50 μM), respectively. The number of spikes discharged by RalBP1$^{-/-}$ neurons in response to the given stimulus intensities was not different from the number of spikes discharged by WT neurons (Fig. 2A and B). In addition, the action potential waveforms of RalBP1$^{-/-}$ neurons, which were evoked by a 2 ms somatic current injection, could be superimposed on the action potential waveforms of WT neurons (Fig. 2C). A quantitative analysis of the action potential waveforms revealed that the reduced expression of RalBP1 in CA1 pyramidal neurons had no effect on the resting membrane potential (+/+, −68.7 ± 0.6 mV; −/−, −69.7 ± 0.8 mV; $p > 0.05$), spike threshold (+/+, −38.0 ± 1.1 mV; −/−, −37.5 ± 1.6 mV; $p > 0.05$), spike duration (+/+, 2.6 ± 0.1 ms; −/−, 2.6 ± 0.1 ms; $p > 0.05$), or spike amplitude (+/+, 116.2 ± 1.2 mV; −/−, 117.9 ± 1.8 mV; $p > 0.05$) of these cells. We further examined the input resistance, a passive membrane property, of the neurons by applying a hyperpolarizing current to CA1 neurons, but we failed to detect any changes in input resistance as a result of RalBP1 reduction (+/+, 140.9 ± 5.8 MΩ; −/−, 143.3 ± 8.7 MΩ; $p > 0.05$, Fig. 2D). Together, these results suggest that the neuronal excitability of RalBP1$^{-/-}$ neurons is intact and that the increased seizure susceptibility of the RalBP1$^{-/-}$ mice is not likely caused by enhanced neuronal excitability.

3.3. Reduced synaptic inhibition in RalBP1$^{-/-}$ CA1 neurons

Because abnormal synaptic transmission in the hippocampus is a risk factor for susceptibility to seizures [17–19], we next investigated if alterations in the synaptic transmission of RalBP1$^{-/-}$ mice produced phenotypes vulnerable to convulsant-induced seizures. Because the spontaneous and evoked excitatory synaptic transmission of RalBP1$^{-/-}$ CA1 neurons did not differ from WT transmission [14], we examined inhibitory synaptic transmission by measuring miniature inhibitory postsynaptic currents (mIPSCs) in the presence of the excitatory postsynaptic current blockers NBQX (10 μM) and D-APV (50 μM), and the sodium channel blocker TTX (0.5 μM). Unexpectedly, we observed fewer inhibitory miniature synaptic events in RalBP1$^{-/-}$ neurons (Fig. 3A). We quantitatively analyzed the amplitudes and frequencies of the mIPSCs recorded in each neuron. As shown in Fig. 3B and C, the amplitudes of mIPSCs were not changed by the RalBP1 mutation (+/+, 12.45 ± 0.37 pA; −/−, 13.24 ± 0.42 pA; $p > 0.05$, Student's *t*-test). However, the frequency of mIPSCs in the RalBP1$^{-/-}$ neurons was significantly lower than the frequency of mIPSCs in WT neurons (6.74 ± 0.33 Hz vs. 5.29 ± 0.30 Hz; $p < 0.01$, Student's *t*-test; Fig. 3D and E). These findings imply that a mutation in the RalBP1 gene impairs synaptic inhibition in CA1 pyramidal neurons.

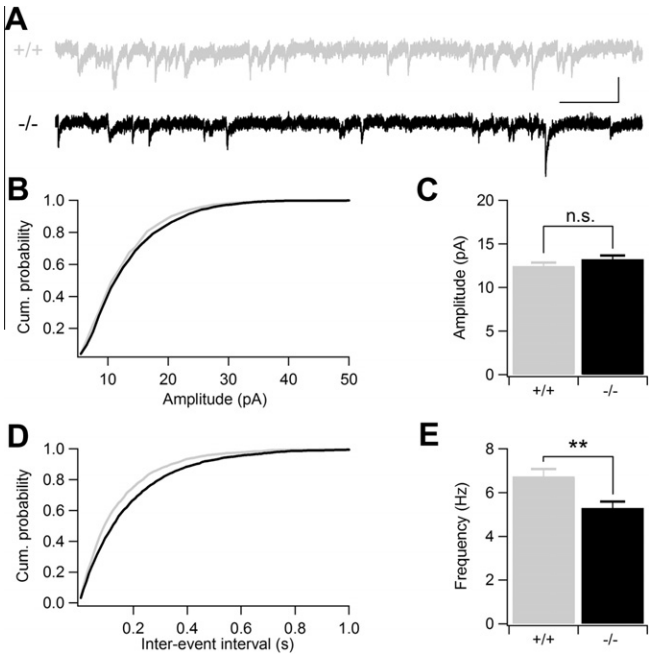

Fig. 3. Reduced RalBP1 expression decreases inhibitory synaptic transmission in CA1 pyramidal neurons. (A) Representative mIPSCs traces recorded in WT and RalBP1$^{-/-}$ neurons. Calibration: 500 ms and 20 pA. (B and C) The mIPSC amplitude was not changed by reduced RalBP1 expression. The cumulative plots (B) and mean mIPSC amplitude values (C) recorded in WT (gray) and RalBP1$^{-/-}$ neurons (black) are shown. n.s., not significant, $p > 0.1$, Student's *t*-test. (D and E) The frequency of mIPSCs was markedly reduced in RalBP1$^{-/-}$ neurons. The cumulative plots (D) and mean mIPSC frequencies (E) recorded in WT (gray) and RalBP1$^{-/-}$ neurons (black) are shown. $^{**}p < 0.01$, Student's *t*-test. (B–E) $n = 25$ neurons from 3 littermates.

3.4. Decreased number of inhibitory neurons in the RalBP1$^{-/-}$ CA1 subfield

The reduced frequency of mIPSCs in RalBP1$^{-/-}$ neurons might stem from a reduced number of inhibitory postsynaptic receptors and/or inhibitory interneurons in the CA1 subfield. Because the majority of inhibitory synapses are located on the dendritic shaft of CA1 neurons [20,21], we examined dendrite arborization in the brain slices of 4–5 week old mice. To visualize the dendritic morphology of individual neurons, we loaded biocytin into CA1 pyramidal neurons using a patch-pipette. Apical and basal dendrites with complex arbors were observed in neurons of both genotypes (Fig. 4A). We quantitatively analyzed the complexity of dendritic branching with a Sholl analysis [22]. As shown in Fig. 4B, the Sholl profiles of the RalBP1$^{-/-}$ neurons were not different from the profiles of the WT neurons. Because some inhibitory terminals innervate dendritic spines in central brain neurons [20,23], we further analyzed spine densities to examine the possibility that dendritic spine innervation differed in the two genotypes. Again, we failed to detect substantial differences in the spine morphology and density of the two genotypes(Fig. 4C and D). These results exclude the possibility that the reduced neuronal inhibition in RalBP1$^{-/-}$ CA1 neurons originates from the impaired development of dendrites or dendritic spines, but indicate the presence of fewer inhibitory neurons in the CA1 subfield [24,25]. We next examined the density of GABAergic inhibitory neurons in the CA1 region with antibodies against glutamic acid decarboxylase 67 (GAD67) [26]. Immunohistochemical staining with anti-GAD67 antibodies revealed GABAergic inhibitory neurons in the CA1 subfields of both WT and RalBP1$^{-/-}$ brains (Fig. 4E). However, the number of GAD67-immunoreactive inhibitory neurons in the CA1 subfield of the RalBP1$^{-/-}$ mice was significantly lower than that of their WT littermates (Fig. 4F–H). These results suggest that the lower number of inhibitory neurons in RalBP1$^{-/-}$ mice reduces the inhibitory input onto CA1 pyramidal neurons.

4. Discussion

RalBP1 is expressed in the mammalian brain, but the behavioral and neurophysiological roles of RalBP1 in vivo are largely unknown. In the present study, we demonstrated that mice hypomorphic for RalBP1 (RalBP1$^{-/-}$ mice) are vulnerable to chemoconvulsant stimulation. The intraperitoneal injection of PTZ induced more severe seizures in RalBP1$^{-/-}$ mice compared to WT mice. We also found that reduced RalBP1 expression decreases the inhibitory synaptic transmission onto CA1 pyramidal neurons and that this impaired synaptic inhibition is associated with the loss of GABAergic interneurons in the CA1 subfield of the hippocampus. Collectively, our data provide direct evidence for the role of RalBP1 in synaptic inhibition and seizure threshold in vivo. In addition, the present study provides new insight into the mechanisms by which small GTPase signaling pathways might be associated with certain types of idiopathic epilepsy.

It was recently proposed that RalBP1 plays a role in drug resistance in epilepsy. However, association between RalBP1 and drug resistance in epilepsy or epilepsy susceptibility is still controversial. A line of RalBP1 mutant mice generated from gene-trapped ES cells (OST 359995) exhibits increased toxicity on phenytoin administration; this phenomenon most likely occurs as a result of decreased drug extrusion mechanisms at the blood–brain barrier [13]. In contrast, genetic studies with epileptic patients have failed to find any association between RalBP1 polymorphisms and drug response or predisposition to epilepsy [27,28]. Our current experiments were limited to the seizure threshold to the convulsant stimulation and related synaptic mechanisms. The current study therefore does not provide any information about the role of RalBP1 in drug transport or drug resistance. However, our data suggest that the reduced synaptic inhibition in RalBP1 hypomorphic mice might produce phenotypes that are vulnerable to convulsant stimulation. Importantly, reduced RalBP1 expression alone was not sufficient to induce epilepsy in mice. When one considers that human epilepsy usually exhibits complex inheritance patterns due to multiple genetic defects and environmental factors, the species-specific influence of different epilepsy mechanisms and differential gene expression profiles in humans and mice might provide one possible explanation for the discrepancy between human and mouse studies [2,4,6]. It should also be noted that our present study was performed on RalBP1 hypomorphs (~18% of WT) generated from a gene-trapped ES cell line (RRC077) rather than RalBP1 null mice. Because gene-trapped mutants can sometimes exhibit tissue-specific skipping or alternative splicing around gene trap insertions [29], native RalBP1 protein might have been produced in this mouse line. On these grounds, we also generated another line of RalBP1 mutant mice with an ES cell line (CSI100) carrying a gene-trap insertion in a different location in the RalBP1 gene. We were able to obtain germ-line chimeras and heterozygous mice but did not observe homozygous mutant mice, suggesting embryonic lethality. Hence, we were unable to further investigate the association between RalBP1 and epilepsy in RalBP1-null mutant mice; this association remains to be investigated in future studies. While the absolute level of RalBP1 activity associated with genetic polymorphisms in the previous studies is unknown [27,28], it is conceivable that a complete loss of RalBP1 function in the brain might cause epilepsy or produce phenotypes that are more sensitive to convulsant stimulation.

We previously reported that RalBP1 mediates AMPA receptor endocytosis during NMDA receptor-dependent long-term depression (NMDAR-LTD) by interacting with the small GTPase RalA

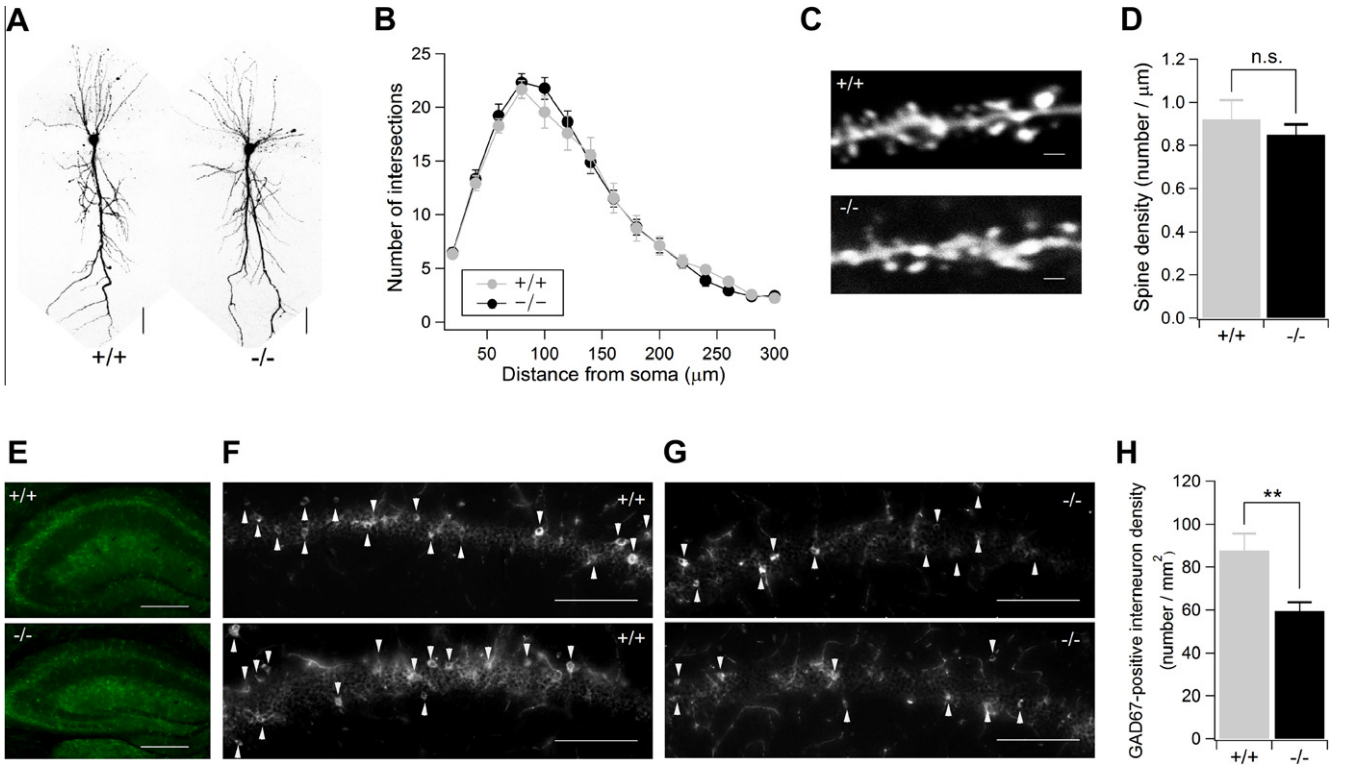

Fig. 4. RalBP1$^{-/-}$ mice exhibit a decreased number of inhibitory interneurons. (A–D) The reduced level of RalBP1 in CA1 pyramidal neurons had no effect on dendritic morphology and spine density. (A) The dendritic morphologies of WT and RalBP1$^{-/-}$ neurons were visualized by biocytin labeling. Scale bars, 50 μm. (B) Sholl profiles of WT and RalBP1$^{-/-}$ CA1 neurons were obtained by counting the number of dendrite branches crossing concentric circles around the cell body. $p > 0.05$, Student's *t*-test. $n = 18$ neurons from 3 littermates. (C) The dendritic spine morphologies of WT and RalBP1$^{-/-}$ CA1 neurons. Scale bars, 1 μm. (D) Quantitative analysis of spine density in WT and RalBP1$^{-/-}$ CA1 neurons. n.s., not significant, $p > 0.1$, Student's *t*-test. $n = 15$ neurons from 3 littermates. (E–H) Loss of inhibitory neurons in RalBP1$^{-/-}$ mice. (E) GABAergic inhibitory neurons in WT (top) and RalBP1$^{-/-}$ (bottom) hippocampi were visualized by immunohistochemical staining with anti-GAD67 antibodies. Scale bars, 500 μm. (F and G) High-magnification images of the CA1 subfield of WT (F) and RalBP1$^{-/-}$ (G) mice. Arrows indicate GAD67-positive inhibitory neurons. Scale bars, 200 μm. (H) Quantitative analysis of GAD67-positive interneuron density in WT and RalBP1$^{-/-}$ CA1 subfields. $^{**}p < 0.01$, Student's *t*-test. $n = 6$ slices from 3 littermates.

and the postsynaptic scaffolding protein PSD-95 [14]. In addition to the role of RalBP1 in the plasticity of excitatory synaptic transmission, the present study demonstrates that RalBP1 is essential for normal inhibitory synaptic transmission. Considering that abnormalities in synaptic function are often associated with neuropsychiatric diseases, behavioral roles of RalBP1 deserve further investigation.

Acknowledgments

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References

[1] B.S. Chang, D.H. Lowenstein, Epilepsy, N. Engl. J. Med. 349 (2003) 1257–1266.
[2] M.H. Meisler, J. Kearney, R. Ottman, A. Escayg, Identification of epilepsy genes in human and mouse, Annu. Rev. Genet. 35 (2001) 567–588.
[3] W.A. Hauser, J.F. Annegers, W.A. Rocca, Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota, Mayo Clin. Proc. 71 (1996) 576–586.
[4] R. Ottman, W.A. Hauser, C. Barker-Cummings, J.H. Lee, N. Risch, Segregation analysis of cryptogenic epilepsy and an empirical test of the validity of the results, Am. J. Hum. Genet. 60 (1997) 667–675.
[5] M.J. Kjeldsen, K.O. Kyvik, K. Christensen, M.L. Friis, Genetic and environmental factors in epilepsy: a population-based study of 11,900 Danish twin pairs, Epilepsy Res. 44 (2001) 167–178.
[6] C.A. Williams, A. Battaglia, Molecular biology of epilepsy genes, Exp. Neurol. (2011), http://dx.doi.org/10.1016/j.expneurol.2011.12.001.
[7] W.N. Frankel, Y. Yang, C.L. Mahaffey, B.J. Beyer, T.P. O'Brien, Szt2, a novel gene for seizure threshold in mice, Genes Brain Behav. 8 (2009) 568–576.
[8] V. Jullien-Flores, O. Dorseuil, F. Romero, F. Letourneur, S. Saragosti, R. Berger, A. Tavitian, G. Gacon, J.H. Camonis, Bridging Ral GTPase to Rho pathways. RLIP76, a Ral effector with CDC42/Rac GTPase-activating protein activity, J. Biol. Chem. 270 (1995) 22473–22477.
[9] V. Jullien-Flores, Y. Mahe, G. Mirey, C. Leprince, B. Meunier-Bisceuil, A. Sorkin, J.H. Camonis, RLIP76, an effector of the GTPase Ral, interacts with the AP2 complex: involvement of the Ral pathway in receptor endocytosis, J. Cell. Sci. 113 (Pt 16) (2000) 2837–2844.
[10] M. Ikeda, O. Ishida, T. Hinoi, S. Kishida, A. Kikuchi, Identification and characterization of a novel protein interacting with Ral-binding protein 1, a putative effector protein of Ral, J. Biol. Chem. 273 (1998) 814–821.
[11] D.F. Kashatus, K.H. Lim, D.C. Brady, N.L. Pershing, A.D. Cox, C.M. Counter, RALA and RALBP1 regulate mitochondrial fission at mitosis, Nat. Cell. Biol. 13 (2011) 1108–1115.
[12] S. Nakashima, K. Morinaka, S. Koyama, M. Ikeda, M. Kishida, K. Okawa, A. Iwamatsu, S. Kishida, A. Kikuchi, Small G protein Ral and its downstream molecules regulate endocytosis of EGF and insulin receptors, EMBO J. 18 (1999) 3629–3642.
[13] S. Awasthi, K.L. Hallene, V. Fazio, S.S. Singhal, L. Cucullo, Y.C. Awasthi, G. Dini, D. Janigro, RLIP76, a non-ABC transporter, and drug resistance in epilepsy, BMC Neurosci. 6 (2005) 61.
[14] K. Han, M.H. Kim, D. Seeburg, J. Seo, C. Verpelli, S. Han, H.S. Chung, J. Ko, H.W. Lee, K. Kim, W.D. Heo, T. Meyer, H. Kim, C. Sala, S.Y. Choi, M. Sheng, E. Kim, Regulated RalBP1 binding to RalA and PSD-95 controls AMPA receptor endocytosis and LTD, PLoS Biol. 7 (2009) e1000187.
[15] R.J. Racine, Modification of seizure activity by electrical stimulation II. Motor seizure, Electroencephalogr. Clin. Neurophysiol. 32 (1972) 281–294.
[16] M.H. Kim, J. Choi, J. Yang, W. Chung, J.H. Kim, S.K. Paik, K. Kim, S. Han, H. Won, Y.S. Bae, S.H. Cho, J. Seo, Y.C. Bae, S.Y. Choi, E. Kim, Enhanced NMDA receptor-mediated synaptic transmission, enhanced long-term potentiation, and impaired learning and memory in mice lacking IRSp53, J. Neurosci. 29 (2009) 1586–1595.
[17] J.O. McNamara, Y.Z. Huang, A.S. Leonard, Molecular signaling mechanisms underlying epileptogenesis, Sci. STKE 2006 (2006) re12.

[18] J.L. Noebels, The biology of epilepsy genes, Annu. Rev. Neurosci. 26 (2003) 599–625.
[19] S.N. Rakhade, F.E. Jensen, Epileptogenesis in the immature brain: emerging mechanisms, Nat. Rev. Neurol. 5 (2009) 380–391.
[20] M. Megias, Z. Emri, T.F. Freund, A.I. Gulyas, Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells, Neuroscience 102 (2001) 527–540.
[21] T.F. Freund, G. Buzsaki, Interneurons of the hippocampus, Hippocampus 6 (1996) 347–470.
[22] D.A. Sholl, Dendritic organization in the neurons of the visual and motor cortices of the cat, J. Anat. 87 (1953) 387–406.
[23] Y. Kubota, S. Hatada, S. Kondo, F. Karube, Y. Kawaguchi, Neocortical inhibitory terminals innervate dendritic spines targeted by thalamocortical afferents, J. Neurosci. 27 (2007) 1139–1150.
[24] I. Cobos, M.E. Calcagnotto, A.J. Vilaythong, M.T. Thwin, J.L. Noebels, S.C. Baraban, J.L. Rubenstein, Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy, Nat. Neurosci. 8 (2005) 1059–1068.
[25] J.C. Gant, O. Thibault, E.M. Blalock, J. Yang, A. Bachstetter, J. Kotick, P.E. Schauwecker, K.F. Hauser, G.M. Smith, R. Mervis, Y. Li, G.N. Barnes, Decreased number of interneurons and increased seizures in neuropilin 2 deficient mice: implications for autism and epilepsy, Epilepsia 50 (2009) 629–645.
[26] C.E. Ribak, J.E. Vaughn, R.P. Barber, Immunocytochemical localization of GABAergic neurones at the electron microscopical level, Histochem. J. 13 (1981) 555–582.
[27] N. Soranzo, L. Kelly, L. Martinian, M.W. Burley, M. Thom, A. Sali, D.L. Kroetz, D.B. Goldstein, S.M. Sisodiya, Lack of support for a role for RLIP76 (RALBP1) in response to treatment or predisposition to epilepsy, Epilepsia 48 (2007) 674–683.
[28] G.D. Leschziner, A.L. Jorgensen, T. Andrew, P.R. Williamson, A.G. Marson, A.J. Coffey, C. Middleditch, D.J. Balding, J. Rogers, D.R. Bentley, D. Chadwick, M.R. Johnson, M. Pirmohamed, The association between polymorphisms in RLIP76 and drug response in epilepsy, Pharmacogenomics 8 (2007) 1715–1722.
[29] A.K. Voss, T. Thomas, P. Gruss, Efficiency assessment of the gene trap approach, Dev. Dyn. 212 (1998) 171–180.