HAP1 interacts with 14–3–3 to regulate epileptic seizure via GABA\_AR-mediated inhibitory synaptic transmission in pentylene tetrazole rat model

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ARTICLE INFO

Key words:
14–3–3
HAP1
GABA\_R\textsubscript{2/3}
Inhibitory synaptic transmission
Epileptic seizure

ABSTRACT

Disruption of γ-aminobutyric acid type A receptors (GABA\_Rs) synaptic clustering and a decrease number in the plasma membrane are thought to contribute to the alteration in the balance between excitatory and inhibitory neurotransmission in the epilepsy. Thus, it is important to elucidate the molecular mechanisms that regulate the stabilities of surface GABA\_Rs populations as well as their concentrations at inhibitory synapses. However, the mechanism that delivers GABA\_Rs to plasma membrane has not been conclusively determined. Our previous research indicated that huntingtin-associated protein 1 (HAP1), a major facilitator of pathological variations in membrane trafficking, modulates epileptic seizure by regulating GABA\_R-mediated inhibitory synaptic transmission in pentylene tetrazole (PTZ)-induced epileptic rats. However, a detailed molecular interaction networks comprising GABA\_Rs and HAP1 is necessary for studying and investigating new treatment targets for epilepsy. In this study, we indicate that HAP1 specifically interacts with 14–3–3, a protein that functions as a chaperone, forming a cargo adaptor complex to regulate surface GABA\_Rs expression and the inhibitory post-synaptic current amplitudes. Disrupting the HAP1/14–3–3 complex decreases the strength of GABA\_R-mediated inhibitory synaptic transmission in epilepsy. Taken together, HAP1/14–3–3 complex is linked to inhibitory synaptic transmission in evoking seizures, therefore, it is a possible drug target for epilepsy.

1. Introduction

Surface γ-aminobutyric acid type A receptors (GABA\_Rs) play an important role in the maintenance of balance between excitatory and inhibitory electrical activities in the central nervous system, which are closely related to the occurrence and development of epilepsy. Therefore, it is important to elucidate the molecular mechanisms that regulate the stabilities of the density of GABA\_Rs at plasma membrane as well as their concentrations at inhibitory synapses. A number of GABA\_R-associated proteins have been identified to be involved in GABA\_R trafficking, and many researchers indicated that mutation and any other changes of those proteins can reduce GABA\_R trafficking and decrease their cell surface expression, which may lead to epilepsy (Sancar and Czajkowski, 2004; Zapata et al., 2017). However, the interaction of GABA\_R-associated proteins for native synapses and trafficking of GABA\_Rs remains to be explored.

The 14–3–3 protein, a so-called “hub protein”, as a chaperone, is a phospho-threonine/phospho-serine binding protein that related with several targets by establishing protein-protein interactions (Fu et al., 2000; Wilker and Yaffe, 2004; Morrison, 2009; Pennington et al., 2018; Stevers et al., 2018). Interestingly, 14–3–3 interacts with GABA\_Rs and facilitate surface GABA\_Rs expression (Nakamura et al., 2016). Our previous research indicated huntingtin-associated protein 1 (HAP1) could attenuate seizure severity in epileptic model and that these alterations are correlated with increased surface GABA\_R\textsuperscript{2/3} expression as well as miniature inhibitory post-synaptic current (mIPSC) amplitudes

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https://doi.org/10.1016/j.neures.2022.05.006
Received 7 January 2022; Received in revised form 23 April 2022; Accepted 18 May 2022
Available online 21 May 2022
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Moreover, another interesting study reported that 14–3–3 regulates HAP1 function by influencing its trafficking in neuronal processes (Rong et al., 2007). However, very few studies have been conducted to analyse the significance of 14–3–3 proteins in neuronal disease, and a detailed mechanism for the interaction of HAP1 and 14–3–3 in regulating GABA<sub>R</sub>α<sub>R</sub>-mediated inhibitory synaptic transmission needs to be further explored. Therefore, we hypothesized that 14–3–3 may also be an adapter protein in the HAP1/GABA<sub>R</sub> complex to form other GABA signalling molecules regulating GABA<sub>ergic</sub> inhibitory synaptic transmission, and this mechanism is involved in epilepsy.

In this study, we first indicated that 14–3–3 expression is reduced in epileptic patients and animal model. Subsequently, we showed that 14–3–3/HAP1/GABA<sub>R</sub>α<sub>R</sub> complex is disrupted in epileptic brain. We then identified that 14–3–3 is involved in epileptic seizure, and this effect is together with HAP1, which is relevant for surface expressions of GABA<sub>R</sub>α<sub>R</sub>, and modulates GABA<sub>ergic</sub> inhibitory synaptic transmission. These results show that the HAP1/14–3–3 complex impacts inhibitory synaptic responses in epileptic and is a novel antiepileptic drug target.

2. Material and methods

2.1. Ethical statement

This study was conducted following the guidelines for the National Institutes of Health of China and the Committee on Human Research at Chongqing Medical University. Written informed consent for human brain tissues were collected. All protocols for the performing animal experiments were admitted by the Commission of Chongqing Medical University for Ethics in Animal Experiments (Approval number:0002648).

2.2. Human samples

Ten temporal cortex samples were obtained from patients with temporal lobe epilepsy (TLE). All the patients were diagnosed with TLE according to International Classification of Epileptic Seizures developed by the International League Against Epilepsy. Ten control cortex samples were obtained from patients with traumatic brain injuries (TBI) without other neurological diseases. The clinical characteristics of control and TLE patients are shown in Table 1 and Table 1 2, respectively.

2.3. Rat model of epilepsy or seizure, treatment and behavioural tests

All Sprague-Dawley rats were obtained from the Laboratory Animal Center of Chongqing Medical University. Chronic and acute pentylenetetrazol (PTZ)-induced epileptic model were established as our previous publication (Li et al., 2020). For chronic PTZ-induced epileptic model, all rats received a subconvulsive dose of PTZ (35 mg/kg, i.p. Sigma) for 30 days. After each PTZ injection, the rats were kept in a Plexiglas chamber and observed for 1 h. The seizure intensity was scored according to the Racine scale (Racine, 1972), which is as follows: 0, no response; 1, facial myoclonus; score 2, head nod, head clonus and myoclonic jerks; 3, unilateral or bilateral limb clonus; 4, double forelimb clonus seizures; and 5, bilateral forelimb clonus with rearing and falling. The rats were considered to be kindled successfully after exhibiting at least three consecutive stage 4 or stage 5 seizures, which were used as epilepsy group, while unsuccessfully kindled rats were used as control group.

For acute PTZ-induced epileptic model, all rats received 70 mg/kg of PTZ (i.p. Sigma) once. LV-GFP, LV-14–3–3 and LV-HAP1 were constructed by GeneChem Corporation (Shanghai, China), and those reagents were stereotactically injected into the bilateral CA1 region of the dorsal hippocampus of each animal in a total volume of 20 μl (10 μl per side) using a glass pipette (0.2 μl/min) 14 days prior to receiving PTZ. BV02, an inhibitor of 14–3–3, was purchased from Sigma, and rats were treated with BV02 (10 mg/kg; i.p.) 24 h prior to receiving PTZ. Picrotoxin (PTX), a GABA<sub>A</sub> antagonist, was purchased from Sigma, and rats were treated with PTX (1 mg/kg; i.p.), which is below the dose range required to induce seizures (>5 mg/kg), 5 min prior to receiving PTZ. Rats were divided into PTZ, LV-GFP+PTZ, LV-14–3–3+PTZ, LV-HAP1+PTZ, Saline+PTZ, BV02+PTZ, LV-14–3–3+PTX+PTZ and LV-HAP1+BV02+PTZ groups according to different treatment. For seizure severity, the behaviours were scored as follows (Ratkai et al., 2012): 0: no further seizures; 1: a generalized clonic seizure; 2: a generalized clonic seizure with loss of righting reflexes; 3: a generalized clonic seizure with loss of righting reflexes plus running and bouncing; 4: all

| No. | Sex (M/F) | Age (years) | Etiology diagnosis | Resected tissue | Pathologic result |
|-----|------------|-------------|--------------------|-----------------|------------------|
| 1   | F          | 22          | Brain trauma       | RTN             | None Normal      |
| 2   | M          | 16          | Brain trauma       | RTN             | None Normal      |
| 3   | M          | 19          | Brain trauma       | RTN             | None Normal      |
| 4   | F          | 27          | Brain trauma       | LTN             | None Normal      |
| 5   | M          | 20          | Brain trauma       | LTN             | None Normal      |
| 6   | F          | 15          | Brain trauma       | LTN             | None Normal      |
| 7   | F          | 9           | Brain trauma       | RTN             | None Normal      |
| 8   | M          | 32          | Brain trauma       | RTN             | None Normal      |
| 9   | F          | 28          | Brain trauma       | LTN             | None Normal      |
| 10  | M          | 22          | Brain trauma       | RTN             | None Normal      |

F, female; M, male; RTN, right temporal neocortex; LTN, left temporal neocortex.

Table 1

Clinical characteristics of patients with TLE.

| No. | Sex (M/F) | Age (years) | Duration | AEDs (years) | Resected tissue | Pathology result |
|-----|------------|-------------|----------|--------------|-----------------|------------------|
| 1   | M          | 31          | 15       | VPA, PB, CBZ, LEV | RTN             | G, NL, ND       |
| 2   | F          | 45          | 20       | CBZ, PHT, VPA, PB | LTN             | G, NL, ND       |
| 3   | M          | 30          | 7        | VPA, PB, CBZ   | RTN             | G, NL, ND       |
| 4   | M          | 25          | 6        | PHT, VPA, PB, TPM | LTN             | G, NL, ND       |
| 5   | M          | 33          | 12       | CBZ, PHT, LTG  | RTN             | G, NL, ND       |
| 6   | M          | 20          | 8        | CBZ, PB, LTG, LEV | RTN             | G, NL, ND       |
| 7   | M          | 15          | 9        | LTG, TPM, CBZ  | RTN             | G, NL, ND       |
| 8   | F          | 21          | 4        | VPA, PB, CBZ   | LTN             | G, NL           |
| 9   | M          | 24          | 5        | CBZ, PHT, PB, LTG | LTN             | G, NL, ND       |
| 10  | F          | 25          | 8        | CBZ, PB, LTG, LEV | RTN             | G, NL, ND       |
behaviours associated with a score of three plus forelimb tonus. Latency was recorded as the time after the PTZ injection to the first seizure onset. The generalized tonic clonic (GTC) value per rat is the number of GTCs per rat within 10 min of PTZ injection. Table 2.

2.4. Stereotactic Injection

Rats were anesthetized and placed in a stereotactic headframe (Reward Life Technology Co., Ltd., Shenzhen, China). Reagents (LV-GFP, LV-14–3–3 or LV-HAP1) were bilaterally targeted to the lateral ventricle (CA1 region: anterior–posterior 3.2 mm, medial–lateral 2.5 mm, and dorsal–ventral 2.6 mm). A microsyringe (Hamilton, Reno, NV) was filled with 10.0 µl of reagents. The needle was inserted into the unilater lateral ventricle, and 10.0 µl of virus was delivered over 10 min. After a 3-min delay, the needle was withdrawn by 0.25 mm, and an additional 10.0 µl of reagents was delivered over an additional 10 min. The injection needle was withdrawn 5 min after the second infusion.

2.5. Tissue preparation

All animals were euthanized with pentobarbital (100 mg/kg, i.p., Sigma, USA) after the seizures were recorded, and cortical and hippocampal tissues were collected. Both the human cortex and rat brain tissues were divided into two parts. One part was stored in liquid nitrogen for Western blot and Co-immunoprecipitation analyses. The other part was fixed in 4% paraformaldehyde for 3 h, successively placed in 20% and 30% graded sucrose solution for 24 h, sliced into 5 µm-thick sections and then stored at −20 °C for immunofluorescence labelling.

2.6. Double immunofluorescence labelling

Immunofluorescence staining was operated as described in our previous study (Li et al., 2020). In brief, sections were fixed in 4% paraformaldehyde for 3 min, then permeabilized with 0.4% Triton X-100 for 15 min, and blocked with donkey serum for 1.5 h. The sections were incubated with a mixture of rabbit anti-14–3–3 (1:50; catalogue number: ab12347; Abcam; UK) and mouse anti-14–3–3 (1:100; catalogue number: 66061–1-Ig; Proteintech; USA) overnight at 4 °C. The next day, the sections were washed three times and incubated with a mixture of donkey anti-rabbit Alexa Fluor 555 (1:50; catalogue number: A0453; Beyotime; China) and donkey anti-mouse FITC (1:50; catalogue number: P2177M; Beyotime; China) according to the manufacturer’s instructions. Input indicated that the samples were pre-treated with nothing, IgG indicated that the samples were pre-treated with A/G agarose and anti-IgG, 14–3–3 indicated that the samples were pre-treated with A/G agarose and mouse anti-14–3–3 (1:50; catalogue number: 66061–1-Ig; Proteintech; USA), GABA β2/3 (1:50; catalogue number: ab39713; Abcam; UK), and HAP1 indicated that the samples were pre-treated with A/G agarose and mouse anti-HAP1 (1:50; catalogue number: ab39713; Abcam; UK). Then, the western blot was performed.

For western blot, SDS-polycrylamide gel electrophoresis separated proteins and those proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. Next, the PVDF membrane was incubated with mouse anti-14–3–3 (1:500; catalogue number: 76601–1-Ig; Proteintech; USA), mouse anti-GABA β2/3 (1:1000; catalogue number: 05–474; Sigma-Aldrich; USA), mouse anti-HAP1 (1:1000; catalogue number: ab39713; Abcam; UK), mouse anti-Glucocorticoid receptor (1:2000; catalogue number: 66240–1-Ig; Proteintech; USA), mouse anti-GAPDH (1:2000; catalogue number: 60004–1-Ig; Proteintech; USA) and rabbit anti-Na+/K+-ATPase (1:1000; catalogue number: 55187–1-AP; Proteintech; USA) overnight at 4 °C. The next day, the PVDF was incubated with HRP-conjugated goat anti-mouse IgG antibody (1:2000; catalogue number: SA00001–1; Proteintech; USA) or HRP-conjugated goat anti-rabbit IgG antibody (1:2000; catalogue number: SA00001–2; Proteintech; USA) for 1.5 h at 37 °C. Densitometry quantitation was detected using the Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, USA).

2.9. Electrophysiological assessments

Whole-cell membrane capacitance patch clamp recordings of mlIPSCs were conducted in pyramidal neurons of the CA1 hippocampal region according to our previous study (Li et al., 2020). Briefly, all electrophysiological recordings were performed in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 1.23 NaHPO4, 2 H2O, 26 NaHCO3, 2 CaCl2, 2 MgCl2, and 10 glucose (pH 7.3–7.4) at 24.5 °C. The membrane potential was sustained at ~60 mV in voltage-clamp mode. mlIPSCs were recorded in the presence of 20 M 6,7-dinitroquinoxaline-2,3 (1 H,4 H)-dione (DNQX), 50 M dl-2-amino-5-phosphonovaleric acid (D-APV), and 1 M tetrodotoxin (TTX). When stable mlIPSCs were obtained, recordings were collected for at least 3 min. The IPSCs were filtered at 2 kHz, digitized at 10 kHz, and analysed using pClamp 9.2 software (Molecular Devices, Sunnyvale, CA, USA) and Mini Analysis software (version 6.0.3; Synaptosoft, Decatur, GA, USA).

2.10. Statistics

Mean comparisons for two groups were done by student’s t test while LSD tests were used for more than two groups. Repeated measures ANOVA was used to compare Sequential measurements and a single control measurement were compared by repeated measures ANOVA, followed by Dennett’s test. p < 0.05 indicated significance.

3. Results

3.1. 14–3–3/HAP1/GABA β2/3 complex is disrupted in TLE patients and in epileptic rats

PTZ-induced epileptic model was established in our study, which is considered an appropriate animal model for preclinical research on the role of GABA β2/3 in epilepsy. We first identified the interaction between 14–3–3 and HAP1, 14–3–3 and GABA β2/3 in the epileptic brain. Immunofluorescence showed that 14–3–3 is co-localized with HAP1 and GABA β2/3 in TLE patients and in epilepsy rats (Fig. 1A, B and C). Additionally, co-immunoprecipitation showed that 14–3–3 is interacted...
with HAP1 and GABA_aRs2/3 in TLE patients and in epilepsy rats (Fig. 1D, E and F). We then detected the level of bounding between 14-3-3 and HAP1, 14-3-3 and GABA_aRs2/3 in the epileptic brain. Compared to the control, co-immunoprecipitation blots and quantification showed that both the levels of 14-3-3 bound HAP1, 14-3-3 bound GABA_aRs2/3 were reduced in TLE patients and in epileptic rats (Fig. 2). These findings suggest the disruption of 14-3-3/GABA_aRs2/3/HAP1 and GABA_aRs2/3 in TLE patients as well as in epileptic rats may be involved in epilepsy.

3.2. 14-3-3 modulates epileptic seizure by regulating GABA_aR function in epileptic rats

Our previous study showed that GABA_aRs2/3 and HAP1 expression were decreased in epileptic brain (Li et al., 2020). In this study, western blot experiments showed that 14-3-3 is decreased in TLE patients and in epileptic rats GABA_aRs2/3 and HAP1 (Fig. 3A, 3B and 3C). We also investigate the expression of surface and total GABA_aRs2/3 and the level of 14-3-3 bound to GABA_aRs2/3, 14-3-3 bound to HAP1, HAP1 bound to GABA_aRs2/3 in the PTZ rats treated with LV-GFP, LV-14-3-3, BV02 and saline. We detected the expression of surface and total GABA_aRs2/3 and found that LV-14-3-3 could not change the levels of GABA_aRs2/3 when compared with LV-GFP (Fig. 3D). However, BV02 could not change the levels of total GABA_aRs2/3, but BV02 could decrease the levels of surface GABA_aRs2/3 when compared with saline (Fig. 3E). Moreover, LV-14-3-3 could increase the level of 14-3-3 bound to GABA_aRs2/3, 14-3-3 bound to HAP1, HAP1 bound to GABA_aRs2/3 when compared with LV-GFP (Fig. 3F). While BV02 could decrease the level of 14-3-3 bound to GABA_aRs2/3, 14-3-3 bound to HAP1, HAP1 bound to GABA_aRs2/3 when compared with saline (Fig. 3G). Those data suggested that up-regulation of 14-3-3 might have anti-epileptic effects through mediating GABA_aR-related mIPSCs in the PTZ-induced seizure rats. Therefore, we detected the change of behaviour and mIPSCs in PTZ-induced seizure rats treated with LV-GFP, LV-14-3-3 or LV-14-3-3 plus PTX (Fig. 3H). The behavioural test indicated that LV-14-3-3 could reduce seizure severity, lengthen latency and decrease GTCs per rat, while those changes were weaken by PTX (Fig. 3I). The LV-14-3-3 could not change the frequency of mIPSCs, but LV-14-3-3 could increased mIPSC, and this change could be weaken by PTX (Fig. 3J).

3.3. 14-3-3, together with HAP1, modulates GABA_aR-mediated inhibitory synaptic transmission in epileptic rats

Our previous research indicated that HAP1 modulates epileptic seizure via GABA_aR function in epileptic rats (Li et al., 2020). Interestingly, 14-3-3 interacts with GABA_aR to facilitate surface GABA_aR

Fig. 1. 14-3-3 is interacted with HAP1 and GABA_aRs2/3. The location of 14-3-3, HAP1 and GABA_aRs2/3 in the temporal cortex of patients with TLE (A), and cortex (B) and hippocampus (C) of epileptic rat. Scale bar = 50 μm (400 ×). The interaction of 14-3-3, HAP1 and GABA_aRs2/3 in the temporal cortex of patients with TLE (D), and cortex (E) and hippocampus (F) of epileptic rat was detected by co-immunoprecipitation.

Fig. 2. 14-3-3/HAP1/GABA_aR complex is disrupted in epileptic brain. Co-immunoprecipitation blots and quantification revealed the levels of 14-3-3 bound to HAP1 was reduced in the temporal cortex of patients with TLE (n = 5, *p < 0.05 vs Control), and cortex (B) and hippocampus (C) of epileptic rat compared to the control (n = 12, *p < 0.05 vs Control). Co-immunoprecipitation blots and quantification showed the level of 14-3-3 bound to GABA_aRs2/3 was significantly reduced in the temporal cortex of patients with TLE (D) (n = 5, *p < 0.05 vs Control), and cortex (E) and hippocampus (F) of epileptic rat compared to controls (n = 12, *p < 0.05 vs Control).
expression (Nakamura et al., 2016), and another interesting study showed that 14–3–3 regulates the functions of HAP1 by influencing its trafficking in neuronal processes (Rong et al., 2007). Thus, we hypothesized 14–3–3, possibly as an adapter in the HAP1/GABA<sub>A</sub>Rs complex, facilitates surface GABA<sub>A</sub>R expression in epileptic brain. To identify this hypothesis, we administered BV02 to rats treated with LV-HAP1 (Fig. 4 A). We found that LV-HAP1 could decrease seizure severity and GTCs per rat (Fig. 4 B), increase surface GABA<sub>A</sub>R<sub>β2/3</sub> expression and the level of HAP1 bound to GABA<sub>A</sub>R<sub>β2/3</sub> (Fig. 4 C), and enhance mIPSCs amplitudes (Fig. 4 D). However, those changes could be weaken by BV02.

4. Discussion

GABA<sub>A</sub> receptors, the targets for various clinical drugs, determine the efficacy of GABA-targeting drugs in TLE patients (Kilman et al., 2002; Naylor et al., 2005; Li et al., 2013; Pavlov and Walker, 2013; Kaila et al., 2014). Understandably, elucidating the cellular mechanisms involved in their accumulation on surface membranes of neurons is of considerable interest. This study showed that HAP1 associate with 14–3–3 is likely to modulate GABA<sub>A</sub>R function by regulating the number of GABA<sub>A</sub>Rs at the cell surface membrane in epilepsy. Reduced membrane GABA<sub>A</sub>R expression relates to enhanced excitation of neuronal circuits and abnormal network oscillations involved.
in epileptogenesis (Kilman et al., 2002; Naylor et al., 2005; Li et al., 2013; Pavlov and Walker, 2013; Kaila et al., 2014). It is widely reported that GABARβ2/3 deficiency decreases GABAR-mediated inhibitory synaptic transmission (Laurie et al., 1992; Wisden et al., 1992; Hentschke et al., 2009; Ren and Curia, 2021). In our study, we found GABARβ2/3 expression was decreased in epileptic brain, suggesting that impaired GABAergic transmission is likely due to reduced β2/3 expression. Moreover, the functional deficiency of HAP1 contributes to reduced surface GABARβ2/3 expression have been reported (Hentschke et al., 2009). Our results from both human and animal model of epilepsy showed that HAP1 was significantly decreased which may be due to dysfunction of 14-3-3 (Hentschke et al., 2009). In our study, we found 14-3-3 upregulation attenuated seizure severity in PTZ rats. Most importantly, we showed 14-3-3 plays a significantly role in HAP1 regulating epileptic seizure, which HAP1 interact with 14-3-3 to regulate surface GABARβ2/3 expression and mIPSC amplitudes. Our results suggest HAP1/14-3-3 complex is accumulated into a big cargo adaptor-motor complex that plays a promoting role in the GABARβR member trafficking attributed to the activity of GABARβR.

Regulation of protein functions require specific PPIs to modulate precise cellular and biochemical functions. Many GABAR receptor-interacting proteins are involved in the clustering, targeting, trafficking, and their stability (González et al., 2015). However, the relation of this interaction for synapses and trafficking of GABARs remains unclear. The 14-3-3 protein, a so-called “hub protein” and an adaptor protein in eukaryotic cells, combines to many other proteins in neuron to modify and regulate target protein functions in various aspects, such as regulation of its molecular interactions, subcellular localizations, enzyme activity, or its stability and structure. At present, more than 200 proteins have been confirmed as 14-3-3 clients (Yam et al., 2012; Riou et al., 2013; Kaplan et al., 2017; Martelli et al., 2018). Notably, some 14-3-3 isoforms are localized at synapses in mature neurons, implying that they involve in synaptic transmission (Baxter et al., 2002), there a study showed that 14-3-3 interacts with GABAR to facilitate surface GABAR expression (Nakamura et al., 2016). However, very few studies have been conducted to analyse the significance of HAP1/14-3-3 in regulating GABAR member trafficking in neuronal disease. In this study, we hypothesized that 14–3–3 is involved in epileptic seizure by regulating GABAR-mediated inhibitory synaptic transmission. We found 14–3–3 upregulation attenuated seizure severity in PTZ rats. Most importantly, we showed 14–3–3 plays a significantly role in HAP1 regulating epileptic seizure, which HAP1 interact with 14–3–3 to regulate surface GABARβ2/3 expression and mIPSC amplitudes. Our results suggest HAP1/14–3–3 complex is accumulated into a big cargo adaptor-motor complex that plays a promoting role in the GABARβR member trafficking attributed to the activity of GABARβR.
is relevant to elevated risk of epilepsy (Tennent et al., 2011). Cerebrospinal fluid (CSF) 14–3–3 levels were reduced in KA-induced epileptic rats (Murphy et al., 2008). De novo 14–3–3 y missense variants lead to epileptic encephalopathies, described by early-onset of epilepsy (Kanani et al., 2020), as well as reduced 14–3–3 levels was observed in rats with focally evoked seizures (Schindler et al., 2006). Phospho-14–3–3 are relevant to neuronal apoptosis in the hippocampus after epilepsy (Kim et al., 2010). 14–3–3 delivery protects against various pathologic changes causing from seizures (Brennan et al., 2013). These results imply 14–3–3 may be a potential target for pharmacological molecular research in epilepsy. In this study, we found 14–3–3 upregulation can attenuate seizure severity in PTZ rats.

5. Conclusions

Taken together, we showed that HAP1, associated with 14–3–3, has a vital function in regulating epileptic seizures. Dysregulated HAP1/14–3–3 complexes involved in abnormal GABA_A Rs member trafficking affects inhibitory synaptic transmission, which led to epilepsy. However, a detailed molecular interaction relating to 14–3–3 molecules, HAP1 and GABA_A Rs are essential for studying.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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