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The effect of exercise on GABA signaling pathway in the model of chemically induced seizures

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ABSTRACT

Aims: Gamma amino butyric acid (GABA) imbalance plays a critical role in most neurological disorders including epilepsy. This study assessed the involvement of mild exercise on GABA imbalance following by seizure induction in rats.

Main methods: Seizure was induced by pentylentetrazole (PTZ) injection. Animals were divided into sham, seizure, exercise (EX), co-seizure-induced exercise (Co-SI EX) and Pre-SI EX groups. In the Co-SI EX group, doing exercise and seizure induction was carried out during four weeks. Animals in the Pre-SI EX group exercised in week 1 to week 8 and seizures were induced in week 5 to week 8. Seizure properties, neural viability and expressions of glutamic acid decarboxylase 65 (GAD65) and $GABA_A$ receptor α1 in the hippocampus were assessed.

Key findings: Seizure severity reduced and latency increased in the Co-SI EX and Pre-SI EX groups compared to seizure group.

The mean number of dark neurons decreased in all exercise groups compared to seizure group in both CA1 and CA3 areas.

The gene level of GAD65 and $GABA_A$ receptor α1 was highly expressed in the Co-SI EX group in the hippocampal area.

Distribution of GAD65 in the both CA1 and CA3 areas increased in the EX and Co-SI EX groups.

$GABA_A$ receptor α1 was up-regulated in the CA3 area of Co-SI EX group and down-regulated in the CA1 and CA3 areas of Pre-SI EX group.

Significance: These findings suggest that exercise develop anti-epileptic as well as neuroprotective effects by modulating of GABA disinhibition.

1. Introduction

Exercise has been considered as an avoidable accepts to improve man's life style. Among various effects of exercise on health, neurological benefits have been illustrated in some researches [1–3]. Both of high and moderate intensity aerobic exercises increase the level of circulatory neurotrophins as well as neurotransmitters and improve psycho-cognitive function in all ages [4–6]. It has been reported that exercise can reduce the chance of dementia, especially Alzheimer's disease and consider as a complementary treatment for depression [7,8]. The older women who had done exercise in the young age demonstrated better cognitive efficiency and lower cognitive deficits in the senility than those who not to do any physical activities [9].

Epilepsy as a common neurological disorder is specified by recurrent and spontaneous seizures [10]. Seizures have been suppressed in > 70% of epileptic patients by anticonvulsant drugs [11]. Some major side-effects such as neurotoxicity and hepatotoxicity of anti-epileptic drugs motivate researchers to find non-drug supplementary treatments [12,13].

Exercise is able to alleviate seizure severity and seizure susceptibility [14]. During exercise, the brain receives abundant signals from various sensory organs that may help to suppress seizure discharges

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[15]. Exercise could increase the β- endorphins release and inhibit epileptic activities [16]. Exercise reduces brain susceptibility to epilepsy before an accelerating brain insult [17].

It is well known glutamic acid decarboxylase (GAD65 and GAD67) has a key role to synthesize GABA in the central nervous system. GAD67 is an active element and produces > 90% of GABA in the central nervous system whereas GAD65 is temporarily activated and enhances GABA levels for fast modulation [18]. GAD67 is more in the cell body and scattered all over the cytoplasm, while GAD65 is abundant in axon terminals [19].

Ionotropic GABA type A receptors ($GABA_A$Rs) are ligand-gated ion channels and work as fast, short-acting synaptic inhibition [20]. $GABA_A$R subunits have been divided into eight classes: α (1–6),β (1–3), γ(1–3), δ, ε, π, θ, and ρ(1–3) [21]. The α subunit protein is a major part to form normal $GABA_A$ receptor [22] and the α_1 subunit is abundant in the adult brain [23].

It has been shown exercise stimulated the γ-aminobutyric acid (GABA) inhibitory system in the hippocampus of epileptic rats [24]. The generation of GABA and GAD in the dorsal horn of mice spinal cord was repaired by treadmill after partial sciatic nerve ligation [25]. The analgesic effect of exercise is partially concerned to the increase of GAD65 [26]. Treadmill exercise has prohibited GABAergic neuronal loss and prevented neuronal activation in the hippocampal CA1 region through the down-regulation of BDNF-TrkB signaling pathway [27].

In this study we assessed the effect of moderate exercise on the hippocampal expression of GAD65 and $GABA_A$ receptor α_1 in the epileptic rats induced by pentylentetrazole (PTZ).

2. Methods

2.1. Animals

Fifty Adult male Wistar rats weighing 250–300 g were housed in the controlled environment with light/dark cycle at 22 ± 1 °C for 1 week before beginning of experiment. All the experiments were carried out according to the protocol approved by the animal ethics of Iran University of Medical Sciences, Tehran, Iran.

Animals were randomly divided into five groups (n = 10, Fig. 1).

1) Seizure group: seizures were induced by intraperitoneal (i.p.) injection of PTZ every other day during four weeks.
2) Sham: normal saline was injected (i.p.) the same protocol of PTZ injection.
3) Exercise (EX): animals were forced to run on a motorized treadmill consisted of 30 min running in 5 days per week for weeks 1–4.
4) Co-seizure-induced exercise (Co-SI EX) group: seizures and EX were carried out in the weeks 1–4. Animals were forced to run on a treadmill 5 days per week and seizures were induced 5 h after doing exercise in the same protocol of seizure group.
5) Pre-SI EX group: animals were forced to exercise like EX group in weeks 1–4 (with no PTZ injection), in week 5–8 seizure and EX were carried out in the same protocol of Co-SI EX group.

2.2. Scoring of convulsive behaviors (seizure severity attacks)

Seizures were induced by i.p. injection of PTZ (35 mg/kg, Sigma Aldrich, Germany). Following by PTZ injections, the convulsive behaviors were observed for 30 min, and resultant seizures were scored as follows: 0 = normal behavior; 1 = immobility; 2 = rigid posture; 3 = repetitive scratching, circling, or head bobbing; 4 = forelimb clonus, rearing, and falling; 5 = repeated occurrence of level four behavior, and 6 = severe tonic-clonic behavior or status epilepticus [28].

2.3. Treadmill exercise protocol

The rats in the three exercise groups (EX, Co-SI EX and Pre-SI EX groups) were forced to run on a motorized treadmill consisted of 30 min running for 5 days per week.

The speed of treadmill was gradually increased (5 m/min every 5 min up to 25 m/min) at 0 degree of inclination [29]. Three minutes were considered to warm up at the beginning and cool down at the end.

2.4. Histological assessment

Five rats of each experimental group in the last day of last week were deeply anesthetized by chloroform and transcardially perfused with 200 ml of saline and 400 ml of 4% paraformaldehyde (PFA) solution. The brains were dissected and inserted in PFA 4% for at least 4 days at 4 °C. Paraffin embedded slices (8 μm-thick coronal sections every 100 μm from 1.8 to 4.3 mm posterior to the bregma) were stained with 0.1% cresyl violet (Sigma Aldrich, Germany) according to the previously described method [27]. Digital camera attached to the microscope (objective lens 40×, Olympus PH-2) was used to capture the images.

Dark neurons (cells with acidophilic cytoplasm and pyknoticnucleus) were counted by Image Tool software (version 3.0) in the hippocampal CA1 and CA3 areas in each specimen (six visual fields/specimen).

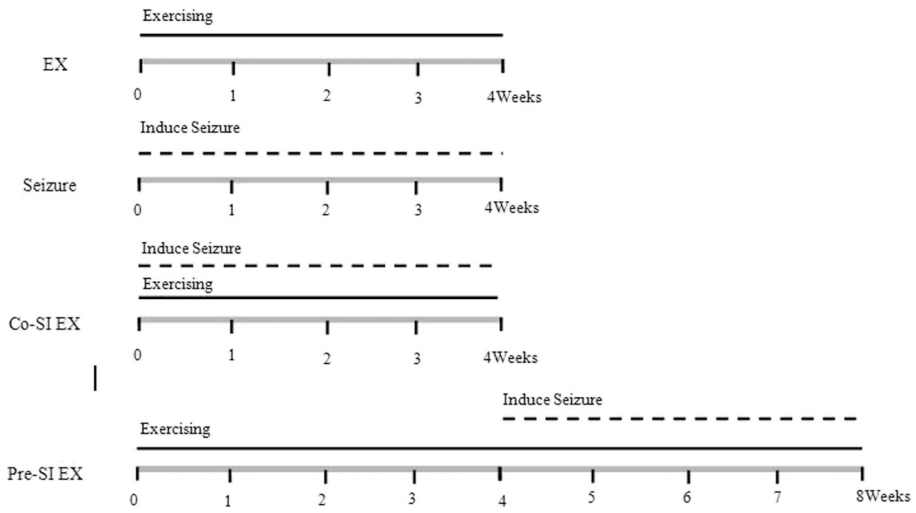

Fig. 1. Schematic plan of exercise and seizure induction.
The solid and dotted lines show the schedule of doing exercise and seizure induction, respectively.

Table 1
Summary of the primer properties.

Name	Forward primer 5′–3′	Reverse primer 5′–3′	Amplicon length (bp)	Annealing temperature (°C)
β-actin NM_031144.3	CGGTCAGGTCATCACTATCGG	ATGCCACAGGATTCCATACCCA	90	60
GAD 65 NM_012563.1	CCAGGCTCATCGCATTCAC	CAAGGATTCTTCTTTCAAGGTCAG	150	58
GABA-A receptor α1 NM_183326.2	GAGAGTCAGTACCAGCAAGAAC	AACACGAAGGCATAGCACAC	148	58

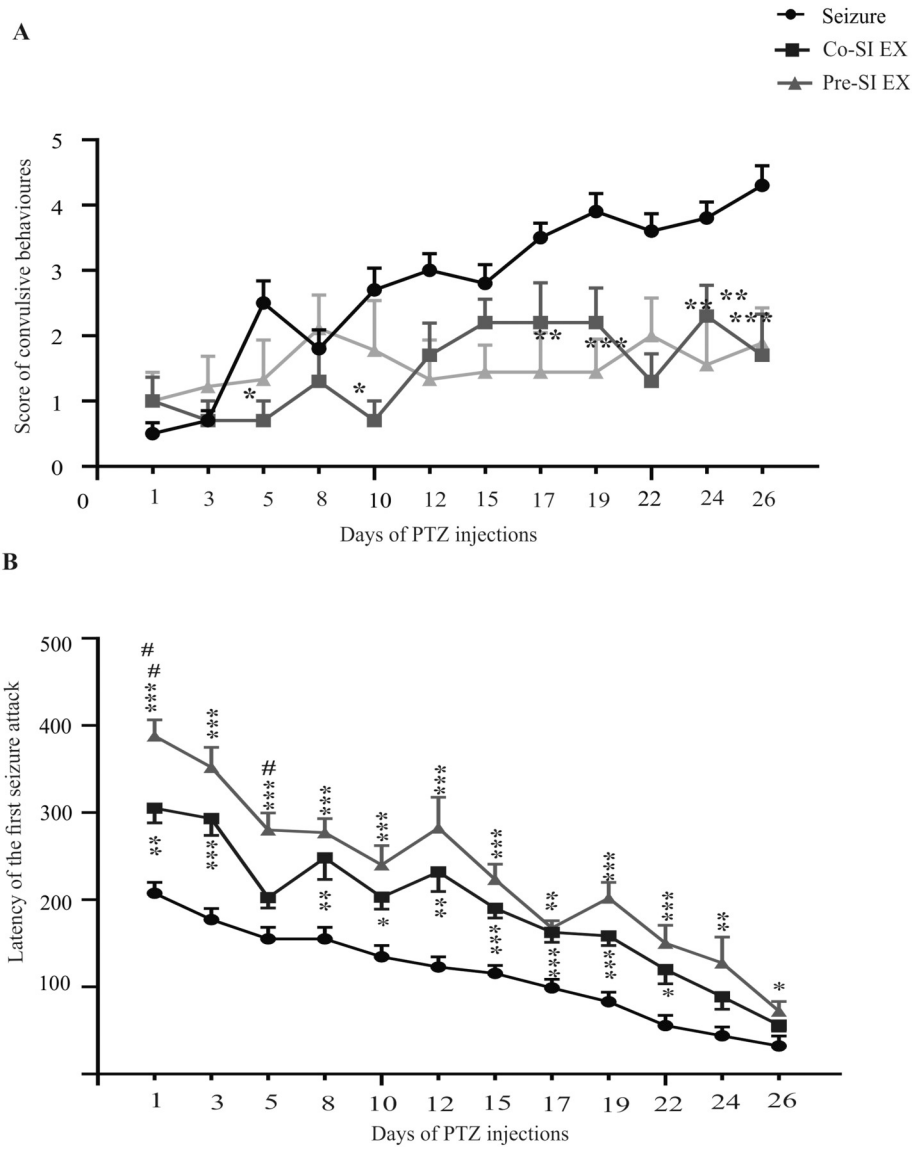

Fig. 2. The effect of exercise on the seizure properties.
A) The graph shows the effect of exercise on the convulsive behaviors which has been scored following by PTZ injection. Data have been shown as the mean ± SEM. Note the significant reduction of scores in Co-SI EX and Pre-SI EX rats compared to seizure group.
B) The graph shows the effect of exercise on the latency of seizures.
The latency of the first seizure attach following by PTZ injection was measured and the mean ± SEM was analyzed. The latency increased in the Co-SI EX and Pre-SI EX groups compared to seizure group.
*, ** and *** indicate $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively compared to seizure group. # and ## indicate $p < 0.05$ and $p < 0.01$ compared to Co-SI EX group.

2.5. Quantitative reverse transcription PCR (qRT-PCR) analysis

Animals were anesthetized by chloroform and perfused with 50 ml cold phosphate buffered saline (PBS) and brains removed ($n = 5$). Hippocampus was dissected in ice and placed in RNAase free tubes and stored at −80 °C. Samples were weighed (range of 30–50 mg) and RNA was extracted from using the RNx-Plus (Sinaclon, Tehran, Iran), according to the protocol, and resolved in 50 µl RNase-free water. The concentration and purity of the isolated RNA was determined by Nanodrop. The denaturing gel electrophoresis method was used to test the RNA integrity. RNA was treated with DNase I (Sinaclon, Tehran, Iran) to eliminate any DNA contamination.

Purified RNA samples were converted into cDNA. cDNAs were synthesized with 1 µg of RNA, 0.5 µl of oligo dTs and 0.5 µl of random hexamer using cDNA Synthesis Kit (Yekta Tajhiz Azma, IRAN). The characteristics of primers were indicated in Table 1. Thermocycling parameters were as follows: initial denaturation at 95 °C for 5 min, 40 cycles of 95 °C for 5 s and annealing and elongation at Table 1 for 20 s. Values from β-actin was used to loading normalization for each sample. Relative changes expression were determined using the $\Delta\Delta Ct$ method relative to gene expression values for control rats (sham group).

2.6. Immunohistochemistry analysis

Three paraffin-embedded sections of each brain were cleared and rehydrated through a series of xylene and alcohol and washed with PBS three times. The sections were boiled in Tris - EDTA buffer at 95 °C for 20 min. After cooling to room temperature for 20 min, the sections were

washed with PBS three times and incubated in blockage solution (1% BSA/PBS 0.1 M for 20 min). The sections were incubated overnight at 4 °C with rabbit polyclonal anti-rat antibodies of $GABA_A$ receptor α_1 and GAD65 (Gene Tex, United State) overnight. Antibodies were diluted 1:100 in 0.1 M PBS at pH 7.4. The sections were then rinsed three times in PBS (10 min each) and incubated with FITC-conjugated goat anti-rabbit secondary antibody (Gene Tex, United State) at the room temperature for 1 h. After washing in PBS three times, the sections were stained by DAPI (Sigma Aldrich, Germany) for identify nucleus. Control for the specificity of immune staining was performed by omission of the primary antibody. Images for analysis were acquired with a digital camera (Nikon, objective lens 40×) attached to the microscope (Nikon Ts100 florescent).

The labelled cells with GAD65 and $GABA_A$ receptor α_1 were assessed by Image Tool software (version 3.0) in the hippocampal CA1 and CA3 areas in each specimen (four visual fields/specimen; ~1 mm^2).

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The criterion for statistical significance was $p < 0.05$.

3. Results

3.1. Effect of treadmill exercise on seizure properties

3.1.1. Seizure severity attacks

To determine of seizure occurrences, the rats were observed fallowed by PTZ injection for 30 min. The mean of scores ± S.E.M in the seizure, Co-SI EX and Pre-SI EX groups were mentioned (Fig. 2A). The severity of seizure attacks in the Co-SI EX group in days of 5 and 10 as well as days of 22 and 26 was significantly reduced compared to the seizure group. ($p < 0.05$ and $p < 0.001$, respectively).

In the Pre-SI EX group, the severity of seizure attacks in days of 19 and 24 as well as 26 significantly reduced compared to the seizure group ($p < 0.01$).

3.1.2. Seizure latency

The latency of the first seizure occurrences following by PTZ injection was measured in the seizure, Co-SI EX and Pre-SI EX groups.

The latency of seizure significantly increased in the Co-SI EX group in days 1, 3, 8, 10, 12,15,17,19 and 22 compared to the seizure group ($p < 0.001$ and $p < 0.01$; Fig. 2B). The mean (*sec*) ± S.E.M was 305 ± 16.7, 293 ± 19.2, 248 ± 24.6, 203 ± 13.9, 232 ± 22.4, 190 ± 11.1, 162 ± 11.2, 158 ± 10.9 and 120 ± 16.3, respectively.

The latency of seizure significantly increased in the Pre-SI EX group in days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24 and 26 compared to seizure group ($p < 0.001$, $p < 0.01$ and, $p < 0.05$; Fig. 2B). The mean (sec) ± S.E.M was 388 ± 18.4, 352 ± 22.8, 280 ± 19.5, 276 ± 16.1, 240 ± 21.9, 282 ± 35.1, 223 ± 17.3, 167 ± 8.2, 202 ± 17.9, 150 ± 20.6, 127 ± 29.6 and 72 ± 10.9, respectively.

Furthermore, the latency of seizure significantly increased in the Pre-SI EX group in days 1 (388 ± 18.4) and5 (280 ± 19.5) compared to the Co-SI EX group ($p < 0.01$ and $p < 0.05$, respectively).

3.2. The effect of exercise on the cell viability

Neurons with acidophilic cytoplasm and pyknoticnucleus (dark neurons), are known as the typical characteristics of morphological changes in the injured neurons. The mean number of hippocampal dark neurons/mm^2 was assessed (Fig. 3B).

The mean number of dark neurons significantly increased in the seizure group (38.67 ± 0.86) as well as Co-SI EX (27.69 ± 0.6) and Pre-SI EX (30.8 ± 1.3) groups in the CA1 area compared to sham group ($p < 0.001$).

In the CA3 area, the mean number of dark neurons increased significantly in the seizure (35 ± 0.66), Co-SI EX (24.67 ± 1.12) and Pre-SI EX (29.61 ± 1.12) groups compared to control group ($p < 0.001$).

Furthermore, the mean number of dark neurons in the CA1 and CA3 areas significantly decreased in the Ex, Co-SI EX and Pre-SI EX groups compared to the seizure group ($p < 0.001$).

3.3. Effects of treadmill exercise on the gene expression of GAD65 and $GABA_A$ receptor α_1

The expression of GAD65 and $GABA_A$ receptor α_1 was quantified by RT-PCR.

The expression of GAD65 increased in the seizure, EX and Co-SI EX group compared to the sham animals ($p < 0.001$). In addition, high expression of GAD65 was shown in the Co-SI EX group while the expression decreased in the Pre-SI EX group compared to the seizure group ($p < 0.001$ and $p < 0.05$, respectively; Fig. 4A).

The $GABA_A$ receptor α_1 expression was increased in the seizure group and Co-SI EX compared to the sham group ($p < 0.05$, $p < 0.001$, respectively; Fig. 3B). $GABA_A$ receptor α_1 highly expressed in the Co-SI EX group compared to the seizure group ($p < 0.01$).

3.3.1. Hippocampal distribution of GAD65

The mean number of immunofluorescence reacted spots has been analyzed/mm^2.

Distribution of GAD65 in the CA1 significantly increased in the seizure (0.74 ± 0.01), EX (0.84 ± 0.009) and Co-SI EX (0.88 ± 0.034) compared to the sham group ($p < 0.05$ and $p < 0.001$, respectively; Fig. 5B).

In addition, GAD65 highly expressed in the EX and Co-SI EX while significantly decreased in the Pre-SI EX (0.66 ± 0.008) compared to the seizure group ($p < 0.01$, $p < 0.001$ and $p < 0.05$, respectively).

Distribution of GAD65 in the CA3 area significantly increased in the seizure (0.68 ± 0.02), EX (0.76 ± 0.01), Co-SI EX (0.942 ± 0.01) and Pre-SI EX (0.689 ± 0.02) groups compared to the sham group ($p < 0.05$, $p < 0.001$, respectively). Furthermore, GAD65 highly expressed in the Co-SI EX compared to the seizure group ($p < 0.001$).

3.3.2. Hippocampal distribution of $GABA_A$ receptor α_1

Distribution of $GABA_A$ receptor α_1 in the CA1 area was significantly increased in the seizure (0.15 ± 0.004), EX (0.14 ± 0.003) and Co-SI EX (0.16 ± 0.003) compared to the sham group ($p < 0.001$ and $p < 0.01$, respectively; Fig. 6B). No significant difference was observed between Pre-SI EX and the sham groups.

Expression of $GABA_A$ receptorα_1 significantly decreased in the Pre-SI EX (0.12 ± 0.001) compared to the seizure group ($p < 0.01$).

Distribution of $GABA_A$ receptor α_1 in the CA3 area was significantly increased in the seizure (0.14 ± 0.003), EX (0.14 ± 0.002) and Co-SI EX (0.16 ± 0.002) compared to the sham group ($p < 0.001$). No significant difference was determined between Pre-SI EX and sham groups.

Expression of $GABA_A$ receptor$\alpha_1$significantly increased in the Co-SI EX while decreased in the Pre-SI EX (0.12 ± 0.001) compared to the seizure group ($p < 0.001$ and $p < 0.01$, respectively).

4. Discussion

4.1. Exercise reduced severity and latency of seizure

We demonstrated that moderate treadmill exercise attenuated the severity of seizures and delayed the initiation of seizure attacks in the epileptic rats. Doing exercise four weeks before initiation of PTZ injection notably decreased seizure severity and increased the latency of the first onset in the early days of seizure induction. However, epileptic animals which had done exercise simultaneously with seizure induction

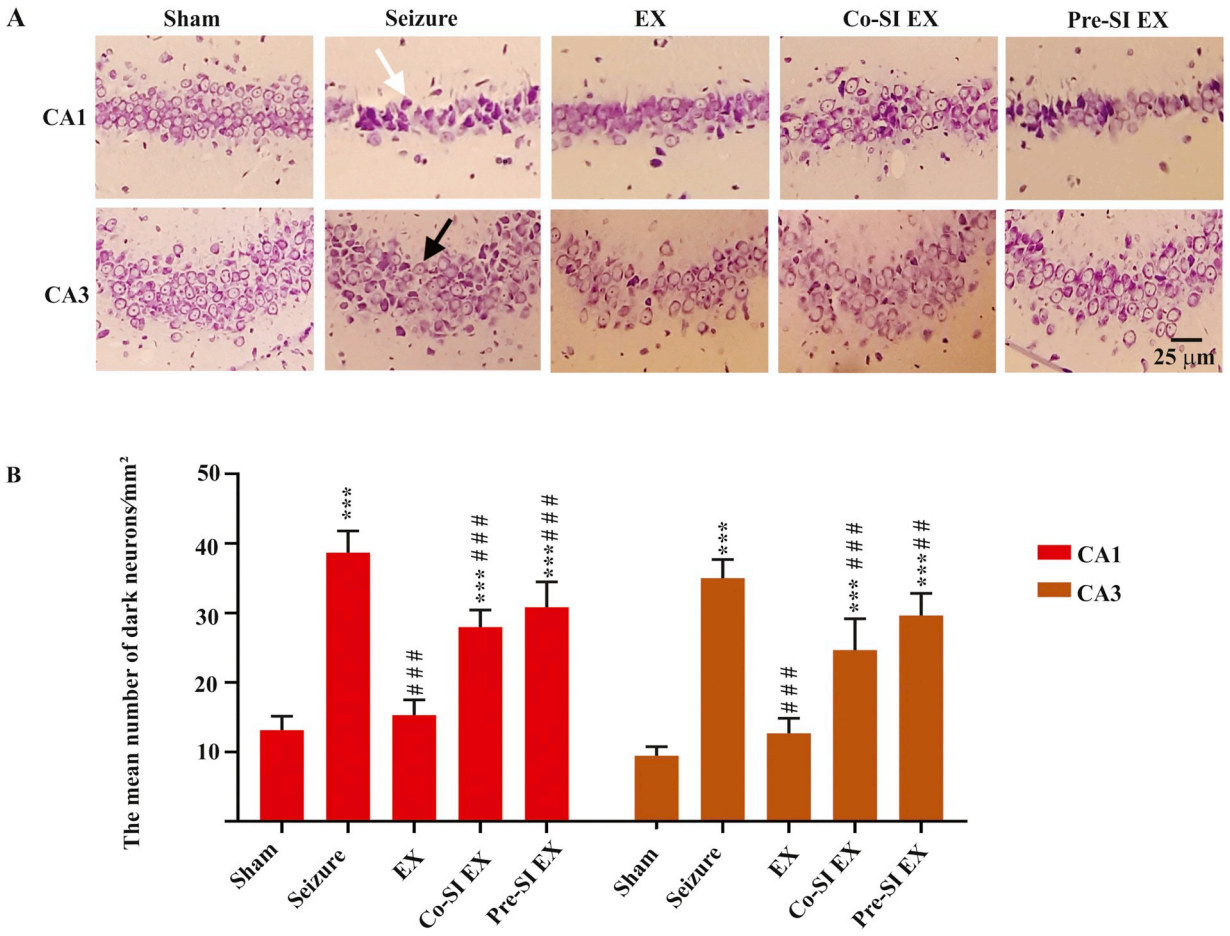

Fig. 3. The effect of PTZ and exercise on the density of dark neurons in the hippocampal CA1 and CA3 areas.
A) Light-microscopic appearance shows dark neurons (white arrow) and normal neurons (black arrow) in the CA3and CA1 hippocampal area. B) The bar graph shows the mean number of dark neurons in the experimental groups. In all exercise groups (EX, Co-SI EX and Pre-SI EX) the mean number of dark neurons was significantly lower than seizure group. ** and *** indicate $p < 0.01$ and $p < 0.001$ compared to sham group. ## and ### indicate $p < 0.01$ and $p < 0.001$ compared to seizure group.

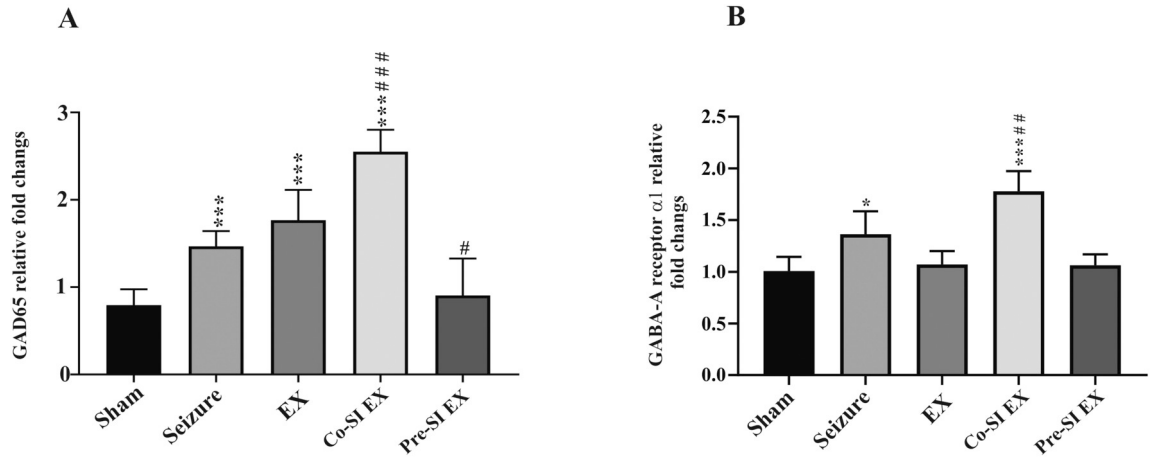

Fig. 4. The gene expression of GAD65 and $GABA_A$ receptor α_1.
A) The bar graph shows the fold changes of GAD65 in the experimental groups. The expression of GAD65 in the seizure, EX and Co-SI EX was significantly increased compared to sham group. The expression of GAD65 in the Co-SI EX was significantly increased and in Pre-SI EX group decreased compared the seizure group.
B) The bar graph shows the fold changes of $GABA_A$ receptor α_1 in the experimental groups.
The expression of $GABA_A$ receptor α_1 in the seizure and Co-SI EX was significantly increased compared to sham group. In addition in the Co-SI EX, $GABA_A$ receptor α_1 highly expressed compared to seizure group.
*, ** and *** indicate $p < 0.05, p < 0.001$, respectively compared to sham group. #, ## and ### indicate $p < 0.05, p < 0.01, p < 0.001$, respectively compared to seizure group.

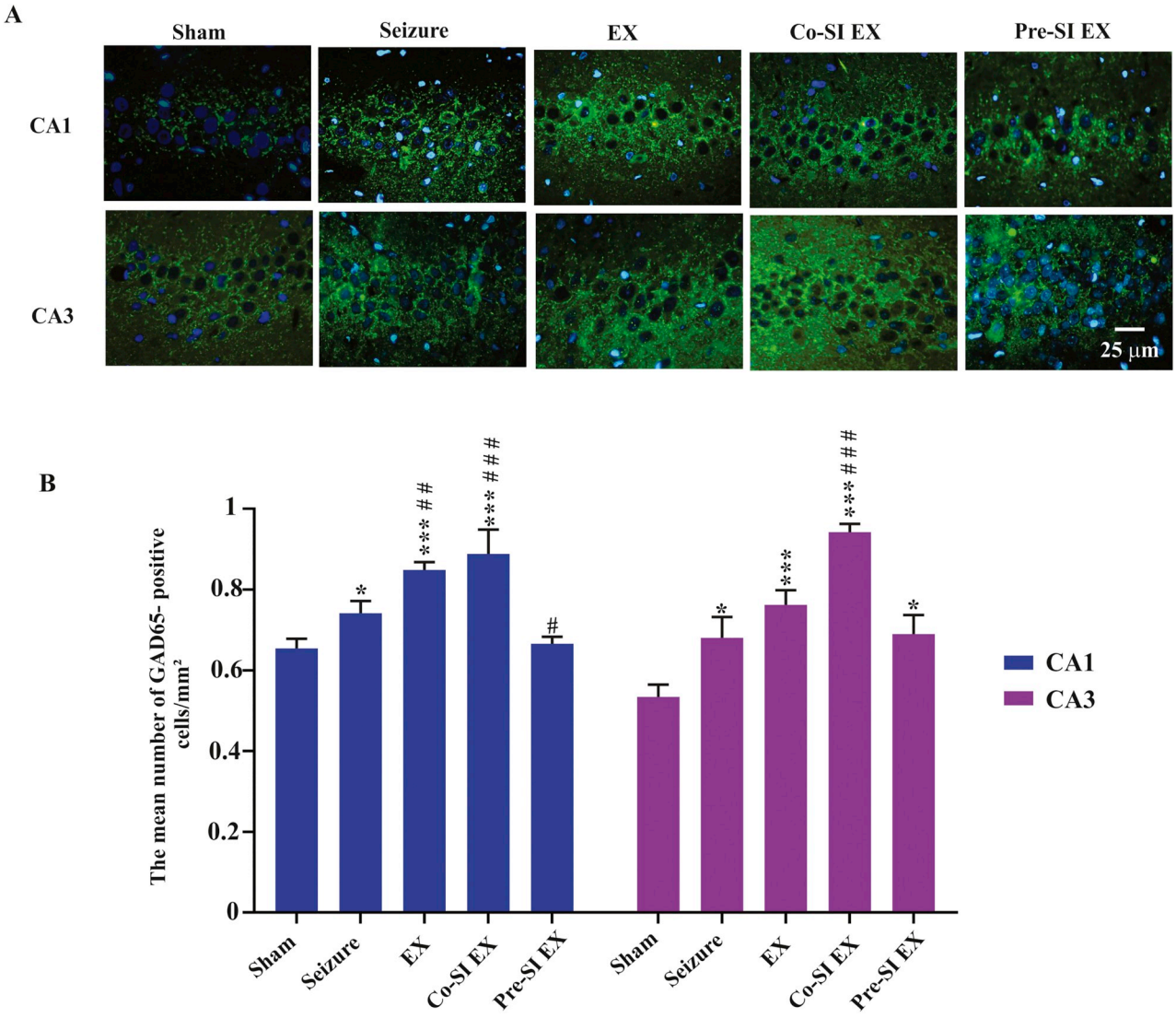

Fig. 5. Distribution of GAD65 in the hippocampal areas.
A) Representative immunofluorescent photomicrographs through the hippocampus of GAD65in green. Nuclei are stained with DAPI in blue.
B) The bar graph shows the mean number green spots per mm^2 ± S.E.M.
Distribution of GAD65 in seizure, EX and Co-SI EX groups the in CA1 andCA3 areas increased compared to sham group. In the CA1 area, GAD65 highly up regulated in the EX and Co-SI EX and down regulated in the Pre-SI EX compared to seizure group.
In the CA3 area, GAD65 highly up regulated in the Co-SI EX compared to seizure group. * and *** indicate $p < 0.01$ and $p < 0.001$, respectively compared to sham group. #, ## and ### indicate $p < 0.05, p < 0.01, p < 0.001$, respectively compared to seizure group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

had been calm down after PTZ injection.

Moderate exercise, as a low-stress exercise, has reduced frequency of seizure attacks in the animal models and epileptic people [30–33]. Doing exercise in early life increased the latency and decreased the severity of seizures in the midlife of rats [34,35].

Evidence of the anti-epileptogenesis aspect of exercise has been emphasized. The aerobic exercise retarded seizure induction in the amygdale kindling model [36]. Enrichment of animal houses delayed epileptogenesis [37]. Physical activity increased resistance to seizure induction in the epileptic model [17]. Our findings showed the latency of the first seizure attack was longer in the animals which had done exercise 4 weeks before induction of seizure in comparison with those did not have any exercise experience before seizure induction.

These findings may suggest the anti-epileptic as well as anti- epileptogenic effect of exercise.

4.2. Neuroprotective effect of exercise

Our findings showed a reduction of dark neuron density in the hippocampal area following by doing exercise during and before seizure induction. The evidences of brain damages and hippocampal sclerosis were identified during the early 19thcentury in epileptic people. Neural injuries have been shown in different brain areas in animal model of PTZ-induced seizure [38].

Pilocarpine-induced seizure highly expressed BDNF and TrkB in the hippocampus [39,40]. In contrary, exercise by down regulation of BDNF and TrkB prevented hippocampal GABAergic neuronal loss [27]. In addition, high level of inflammatory factors such as IL-6, IL-1β and TNF-α that has been shown in the epileptics have important role in the neural hyper-excitability and toxicity [41,42]. Physical exercise can modulate the level of IL-6 and TNF-α in the blood circulation [43,44].

Furthermore, neural death increased following by reduction of parvalbumin as a marker of inhibitory interneurons that has a great

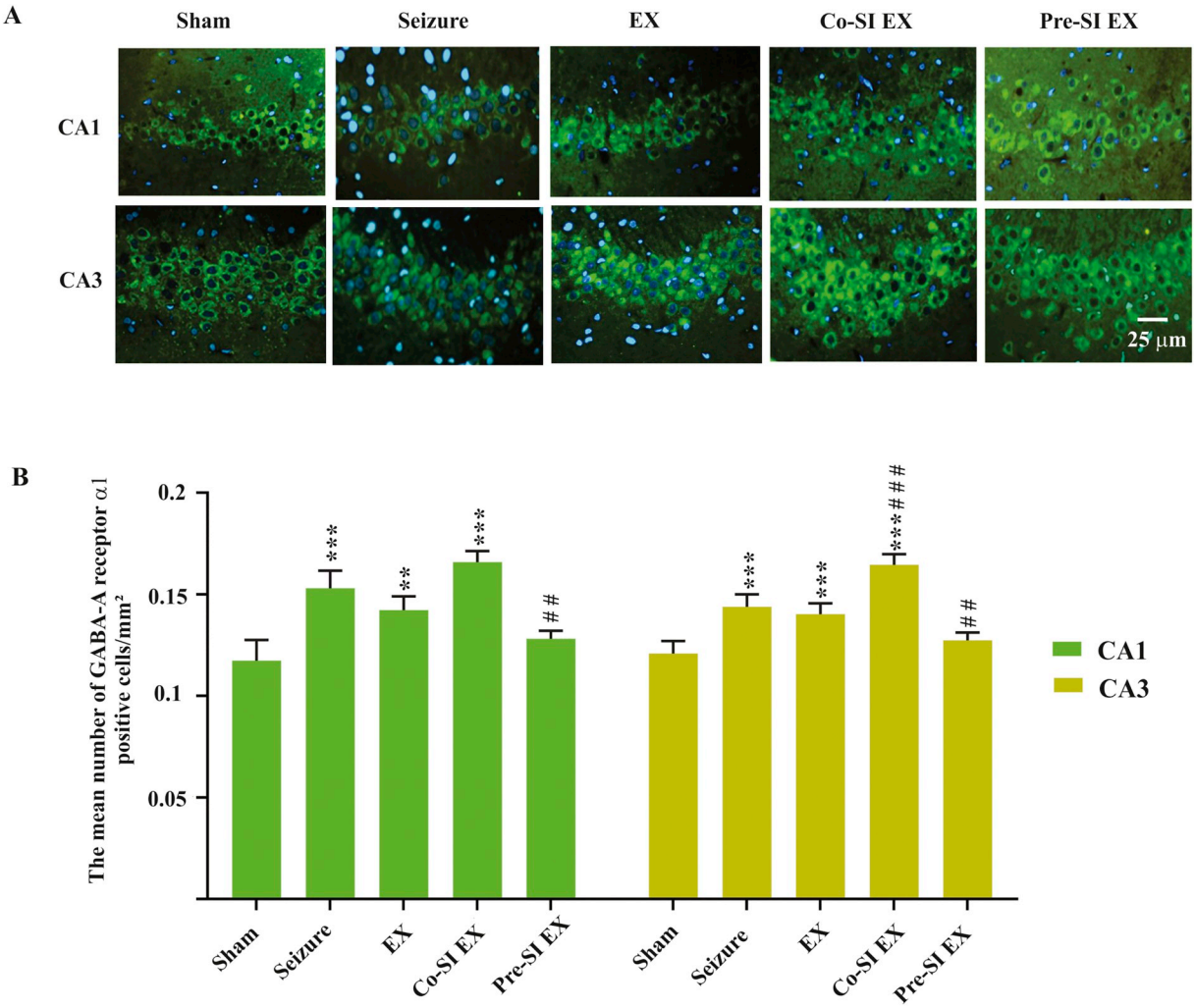

Fig. 6. Distribution of $GABA_A$ receptor $\alpha_1$in the hippocampal areas.
A) Representative immunofluorescent photomicrographs through the hippocampus of $GABA_A$ receptor $\alpha_1$in green. Nuclei are stained with DAPI in blue.
B) The bar graph shows the mean number of reacted cells per mm^2 ± S.E.M.
This receptor highly up regulated in the seizure, EX, Co-SI EX groups in both CA1 and CA3 areas compared to sham group and down regulated in Pre-SI EX compared to seizure group. ** and *** indicate $p < 0.01$ and $p < 0.001$, respectively compared to sham group. ## and ### indicate $p < 0.01$ and $p < 0.001$, respectively compared to seizure group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

deal of interruption with calcium [45]. Exercise increased the number of parvalbumin cells in the hilus of dentate gyrus [46].

This evidence according our findings suggests the neuroprotective effect of exercise may due to clearing of inflammatory factors as well as survive the GABAergic neurons.

4.3. Exercise modulated GABA signaling pathway in the epileptic conditions

We showed that epileptic animals with no experience of exercising expressed high level of GAD65 and α_1 subunit of $GABA_A$ receptors.

According to our data, it has been shown high expression of α_1 subunit in the CA1, dentate gyrus and CA3 regions in the epileptic animals [47]. Temporary reduction in mRNA levels in all of α subunits, β_2, β3 and γ_2 subunits has been reported in the proximal subiculum and entorhinal cortex 24 h after kainic acid injection while 30 and 90 days after same injection, α_1 mRNA increased in the hippocampus and superficial entorhinal cortex [48]. In addition, α_1 and γ_2 subunits immunoreactivities decreased during status epilepticus [49,50].

It seems that high expression of GAD65 as well as $GABA_A$ receptor α_1 following by PTZ injection would be a compensatory response to chemically induction of seizures. Enhanced- expression of GAD within the interneurons and granule cells/mossy fibers suggests self-protection mechanisms in the epilepsy [51].

Furthermore, we indicated the synergic effect of exercise on the high expression of GAD65 and $GABA_A$ receptor α_1 in the animals which had done exercise simultaneously with seizure induction. Several studies indicated that exercise has improved GABA signaling pathway in different brain areas [25,27,52].

It is well known that the most important causes of epilepsy can be pointed to inflammatory factors such as IL-6, IL-1β and TNF-α [41,53]. Most of inflammatory factors inhibit the function of the $GABA_A$ receptor in the hippocampal neurons and lead to the hyper excitability of the nervous system [54]. It has been reported that physical exercise can modulate the level of the most cytokines in the blood circulation [43,44].

It seems that improvement of GABA signaling pathway developed the anti-epileptic effect of exercise.

In addition, we showed seizure severity as well as neural survival was improved in both Co-SI EX and Pre-SI EX groups. However, the expression of GABA and GAD increased in the Co-SI EX, not in the Pre-SI EX group. It should be mentioned that high expression of GABA and GAD was seen in the health animals which did exercise. It seems that long-lasting of exercising (4 weeks before induction of seizures) enhanced GABA signaling resulted in modulating of GABA imbalances

following by PTZ injection.

The low hippocampal level of GAD65 increased seizure susceptibility in the Mongolian gerbil [55]. Deficit of GAD65 in mice triggered seizure occurrences [56]. Reduction in the expression of GAD65 and 67 in the cortical layer V had involved in the epileptogenesis [57].

In addition, $GABA_A$ α_1 subunits are involved in the epileptogenesis. It may be directly exposed to GABA oscillations which are caused by seizures or any pathological conditions [47].

It has been shown that down-regulation of $GABA_A$ receptors in different brain areas has crucial role to develop status epilepticus [58,59].

In the animal model of temporal lobe epilepsy, hippocampal $GABA_A$ receptor$\alpha_1$enhanced in mean time of seizure-free time and decreased following by development of epileptogenesis [60].

It seems that high expression of the inhibitory GABA receptors in the hippocampus of animals which exercised during seizure induction improved the GABA deficit following by PTZ injection (as a GABA blocker). Our findings suggest that exercise could maintain the inhibitory/excitatory equilibrium in the epileptic conditions.

5. Conclusion

The potential anti-epileptogenesis as well as anti- epileptic effect of exercise could be concluded. In addition, these findings suggest the moderate exercise as a supplementary treatment for epilepsy.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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