Epilepsy Research 157 (2019) 106208

Contents lists available at ScienceDirect

Epilepsy Research

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

Effects of vitamin D and paricalcitol on epileptogenesis and behavioral properties of WAG/Rij rats with absence epilepsy

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ARTICLE INFO

Keywords:
Absence epilepsy
Paricalcitol
Vitamin D
Seizure
WAG/Rij rat

ABSTRACT

Aim: Vitamin D (Vit D) has been considered as a neurosteroid and has a pivotal role in neuroprotection including epilepsy. Vit D regulator acts via a Vit D receptor (VDR). WAG/Rij rats have a genetically epileptic model of absence epilepsy with comorbidity of depression. The aim of the present study was to investigate the effect of Vit D and paricalcitol (PRC) on WAG/Rij rats.

Material and methods: Sixty-three male WAG/Rij rats and seven male Wistar rats were used. The effects of acute and chronic treatment with Vit D (5.000 and 60.000 IU/kg, i.p) and PRC (0.5, 5 and 10 μg/kg, i.p) on absence seizures, and related psychiatric comorbidity were investigated in WAG/Rij rats. Depression-like behavior was assayed by using the forced swimming test (FST) and; anxiety-like behavior by using the open field test (OFT).

Results: Acute Vit D treatments (5.000 and 60.000 IU/kg) similarly reduced the number and duration of spike-wave discharges (SWDs) and showed anxiolytic-antidepressive effect whereas there were no significant changes in other measured parameters between the daily and the bolus dose of Vit D. Acute administration of PRC (0.5, 5 and 10 μg/kg) showed anti-convulsive and anxiolytic-antidepressive effect. The dose (0.5 μg/kg) of PRC was the most effective dose. Chronic treatment was more effective than acute therapy in all parameters.

Conclusion: The results of the present study demonstrate that Vit D and PRC have antiepileptic and anxiolytic-antidepressive effects on the absence epilepsy in WAG/Rij rats.

1. Introduction

Epilepsy is one of the most common neurologic diseases characterized by seizure episodes affecting the nervous system and causing mental and physical dysfunction. Depressive disorders including depression and anxiety are the highest prevalence psychiatric comorbidity in patients with epilepsy (Tellez-Zenteno et al., 2007). Clinical and animal studies indicate that depression is accompanied by many types of epilepsy including absence epilepsy (Hesdorffer et al., 1998; Kanner and Balabanov, 2002; Mula and Schmitz, 2009; Trinka et al., 2006; Sarkisova and van Luijtelaar, 2011; Aygun et al., 2019).

Vitamin D (Vit D) plays a significant role in body functions including the nervous system. Calcium homeostasis acts in the regulation of the nervous system and may increase the synthesis of neurotransmitters, including norepinephrine, dopamine, and GABA which plays a key role in releasing the brain inhibitory neurotransmitters and regulation of epileptiform activity (Carswell, 1997; Garcion et al., 2002). Paricalcitol (PRC) is a Vit D receptor agonist (VDR). It is known that the pleiotropic effects of VDR activation are responsible for the distribution of VDR all over the human body's nervous system, intestine, kidney, bone, parathyroid gland, immune system, and myocardium. In particular, VDR is widespread in the brain and the spinal cord, including the areas involved in regulation of motor activity and behavior (Prufer et al., 1999; Langub et al., 2001; Walbert et al., 2001; Fedotova, 2017).

Absence epilepsy is one of the most common types of pediatric epilepsy, occurring among 10 and 17% of all cases of childhood-onset epilepsies (Berg et al., 2000). Absence seizures can also be seen in adults but are relatively rare. It is frequently seen between the ages of 6-12. Vit D deficiency is common in patient with epilepsy especially in children (Teagarden et al., 2014). A lot of studies reported that the prevalence of Vit D insufficiency or deficiency in children with epilepsy ranged between 50–66% in USA, Australia, Denmark, Turkey, Israel, Spain, Iran, Korea and Egypt, Africa (Vestergaard et al., 2004; Lee et al., 2015; Fong et al., 2016; Yaghini et al., 2015; Hasaneen et al., 2017; Yildiz et al., 2017; Sreedharan et al., 2018; Durá-Travé et al., 2018; Inaloo et al., 2019; Kija et al., 2019). Recent studies have shown the therapeutic potential of Vit D use in many neurological disorders

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https://doi.org/10.1016/j.eplepsyres.2019.106208
Received 1 June 2019; Received in revised form 10 August 2019; Accepted 14 September 2019
Available online 19 September 2019
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including epilepsy and depression (Uyanıkgil et al., 2016; Liang et al., 2017; Durá-Travé et al., 2018). Several experimental studies demonstrated that Vit D treatment decreased electrical hippocampal seizures in rat (Siegel et al., 1984), pentylenetetrazole (PTZ)-induced convulsions in mice (Kalueff et al., 2005a, 2005b) and produced anticonvulsant activity in PTZ-kindled rats (Abdel-Wahab et al., 2017). However, there is no data about the effects of Vit D and Vit D receptor agonist PRC on absence seizures.

Wistar derived inbred line, the WAG/Rij rats are useful genetic animal models of absence epilepsy and are reported to have different behavioral properties compared to Wistar rats. Genetically epileptic WAG/Rij rats develop spontaneous absence-like seizures after 3 months of age (Kanner and Balabanov, 2002). Most of the studies providing evidence that WAG/Rij rats decreased both investigative activity and locomotor activity in the open field test (OFT) (Cotman and Berchtold, 2002; Sarkisova et al., 2010), increased immobility time and decreased swimming time in the forced swimming test (FST); therefore, WAG/Rij rats are generally recognized as a good model of genetic absence epilepsy with comorbid depressive-like symptoms (Sarkisova et al., 2010; Sarkisova and van Luijtelaar, 2011; Russo et al., 2011, 2013; Citraro et al., 2015).

The aim of the present study was to evaluate the effects of Vit D and PRC on different behavioral states related with locomotor activity, anxiety, depression and absence seizures effect in WAG/Rij rats by electrocorticography (ECoG), OFT and FST.

2. Materials and methods

2.1. Animals

Six-three months old male of inbred WAG/Rij (n = 63) and non-epileptic control rats (outbred Wistar; n = 7), weighing between 200–250 g were used in the experiments. All animals were kept under standard 12-h light/dark cycle in a temperature-controlled (22 ± 2 °C) environment with ad libitum access to rodent chow. Before starting the experiments, all rats were adapted to the laboratory conditions for at least one week. All studies were approved by the Gaziosmanpasa University Local Ethics Committee on Animal Experiments (2018/17-36) and were in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2. Surgical procedure

Before the surgery, all animals were anesthetized and sedated with ketamine (100 mg/kg, i.p) + xylazine (10 mg/kg, i.p) intraperitoneally (i.p). Animals were placed in stereotaxic apparatus; the skin and subcutaneous tissue were lifted from the cranium, and the skin was folded back. Three small burr holes were drilled with a microdrill (Fig. 1A) without damaging the dura mater and bipolar stainless steel recording electrodes and reference electrode (ground electrode), (0.12 mm diameter, Plastic products company, MS 333/2A) were inserted on the cortex (Fig. 1B). Stainless-steel electrodes were placed on the dura mater over the cortex: one in the frontal region (2 mm anterior and 3.5 mm lateral to bregma) and parietal region (6 mm posterior and 4 mm lateral to bregma) according to the atlas coordinates of Paxinos and Watson (1986). A reference electrode was implanted over the cerebellum. Electrodes were fixed to the skull with dental cement (Fig. 1C). Then scalp was sutured (1D), (Aygun et al., 2015, 2019).

2.3. Electrophysiological recordings

Following the surgical procedure, each animal was placed in separate cages and left for a week as a healing period. After seven days of healing, rats were placed individually in a registration cage (30 × 30 cm in width, 50 cm high) and connected to recording leads. In the first set of experiments, all animals were taken for an ECoG recording of three hours. All three hours of ECoG recordings were recorded at the same time of day (09:00 AM) using AcqKnowledge software (version 3.8) and the MP-150 multichannel physiological analysis system (BIOPAC Systems Inc., Goleta, CA, USA) from free-moving animals in a noise-isolated room (Fig. 2B). After the drug injection rats were connected to recording leads again and ECoGs recorded for 3 h. The total number, the total duration, and the amplitude of the spike-wave discharges (SWDs) were calculated offline.

2.4. Drugs and drug administration

Ketamine hydrochloride (HCl) and xylazine hydrochloride, (Sigma Chemical Co., St. Louis, MO, USA), paricalcitol (Zemplar, Abbott) and Vit D (purchased from Abdi İbrahim) were used. The doses of Vit D and PRC were determined in accordance with previous studies (Mehta et al., 2014; Sinha et al., 2013; Gul and Aygun, 2019).

In the first set of experiments, baseline ECoG recordings were obtained for all groups. In the second set of experiments, acute treatment of Vit D and paricalcitol following as; a single doses of Vit D (5.000 and 60.000 IU/kg by intraperitoneal injection (i.p) and a single doses of PRC (0.5, 5 and 10 µg/kg/i.p,) was administered and then ECoG recording and behavioral test (OFT and FST, respectively) were taken. In the third set of experiments, chronic treatment of Vit D and paricalcitol following as; chronic Vit D (5.000 IU/kg, daily for 2 weeks, or and

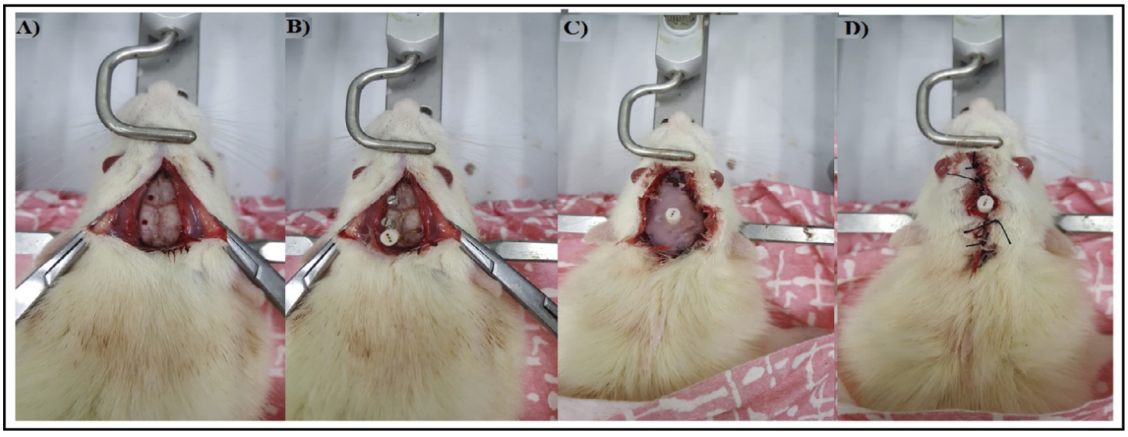

Fig. 1. A) Animals were placed in a stereotaxic apparatus; the skin and subcutaneous tissue were lifted from the cranium, then micro-drilling of the skull where the electrodes are inserted. B) Tripolar electrodes and steel screw were placed on the skull. C) The skull was closed with the help of dental acrylic to stabilize the tripolar electrodes and screws. D) The scalp was sutured.

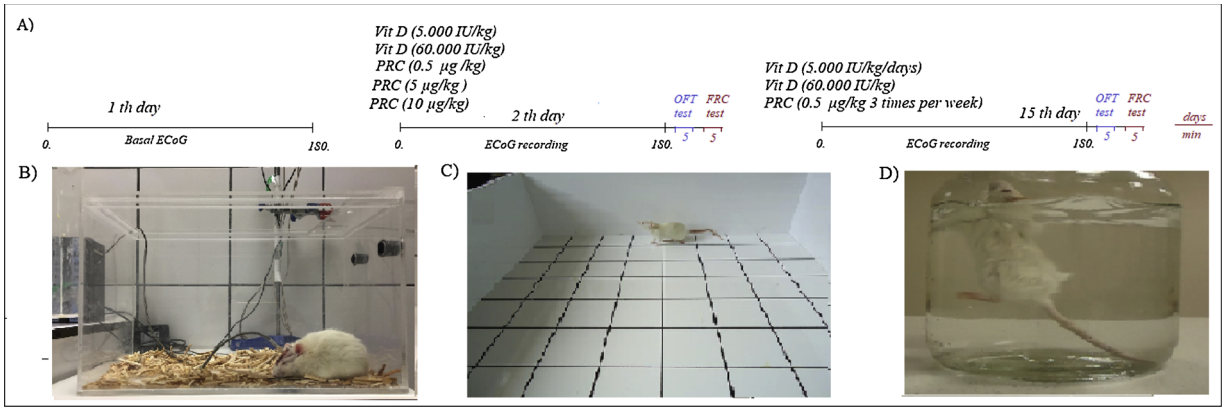

Fig. 2. (A) Experimental timeline (B) electrophysiological recording (C) open field test application (D) forced swimming test application.

60.000 IU/kg, bolus dose, single dose) and effective doses of PRC (0.5 µg/kg, three times per weeks, for 2 weeks) were administered. ECoG recording and behavioral test (OFT and FST, respectively) were taken at 15th day.

Animals were divided into the following experimental groups:

1 Non-epileptic Wistar rat group (This group will be used for behavioral comparison between Wistar and WAG/Rij rats)
2 WAG/Rij rat group
3 WAG/Rij rat + Vitamin D (5.000 IU, acute) group,
4 WAG/Rij rat + Vitamin D (5.000 IU, chronic) group
5 WAG/Rij rat + Vitamin D (60.000 IU, acute) group
6 WAG/Rij rat + Vitamin D (60.000 IU, chronic) group
7 WAG/Rij rat + Paricalcitol (0.5 µg, acute) group
8 WAG/Rij rat + Paricalcitol (0.5 µg, chronic) group
9 WAG/Rij rat + Paricalcitol (5 µg, acute) group
10 WAG/Rij rat + Paricalcitol (10 µg, acute) group,

2.5. Evaluation of anxiety-like behaviors

2.5.1. Open Field Test

All animals were tested in the open field apparatus which consisted of a 100 x 100 cm square arena divided into 64 equal segments and surrounded by a wall of 30 cm high. The behavioral properties of the animals were evaluated in an open field arena over a five minutes period. The movements of the animals were recorded by a video camera. The test started by placing the animal in the center of the square arena. During the test, the number of squares crossed, the number of rearings and duration of grooming were analyzed (Fig. 2C), (Aygun and Gul, 2018).

2.6. Evaluation of depression-like behavior

2.6.1. Forced swimming test

Rats were placed individually into glass cylinders (height: 45 cm, diameter: 30 cm) containing 20 cm of water, maintained at 22–23 °C. Test sessions were recorded by a video camera. The duration of immobility time (passive swimming time) and the duration of swimming time were measured for five minutes. The criterion for immobility time or passive swimming time was floating vertically in the water while making only those movements required in order to keep rat's head above the water. The criterion for swimming time was strong movements of all four limbs, jumping, struggling, thrashing and climbing on the glass cylinders wall (Porsolt et al., 1977; Lahmame et al., 1997; Lucki, 1997). After the forced swimming test (FST), rats were dried with a towel, then put in its own cages (Fig. 2D).

2.7. Statistical analysis

Statistical analysis was performed with the SPSS 15.00 program. Paired-Samples *T*-test was applied between two dependent groups in comparison with baseline records of the groups. One-way ANOVA and post hoc LSD test were used to compare Vit D, PRC, and post-sham injection groups. Student's *t*-test was used on non-epileptic Wistar rats group compared with WAG/Rij (control) rats group behavior parameters. The results are expressed as mean ± standard error (SEM). For all statistical tests, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Evaluation of electrophysiological recordings

In 6-month-old WAG/Rij rats, repetitive typical spike-wave discharges consisting a total number of 81.0 ± 5.30 and duration of SWDs

Table 1
Effects of Vitamin D and paricalcitol on ECoG parameters in WAG/Rij rats.

Groups	The Number of SWDs	The Duration of SWDs (sec.)	The Amplitude of SWDs (µV)
WAG/Rij	81.0 ± 5.30	444.0 ± 24.98	558 ± 20
Vit D (5.000 IU, A)	52.16 ± 6.22,**	244.3 ± 28.67,***	589 ± 17
Vit D (5.000 IU, C)	41.56 ± 4.33,***	199.3 ± 18.90,***	539 ± 42
Vit D (60.000 IU, A)	52.23 ± 5.45,**	275.0 ± 22.34,***	575 ± 30
Vit D (60.000 IU, C)	36.67 ± 5.71,***	204.4 ± 34.56,***	523 ± 37
PRC (0.5 µg, A)	37.5 ± 2.12,***	230.0 ± 27.46,***	594 ± 20
PRC (0.5 µg, C)	43.20 ± 3.27,***	239.4 ± 27.46, ***	586 ± 12
PRC (5 µg, A)	50.5 ± 4.01, ***	255.5 ± 9.03, ***	546 ± 23
PRC (10 µg, A)	67.16 ± 5.23	270.2 ± 13.96, ***	567 ± 41

Data are presented as mean ± SEM. One-way ANOVA and multiple comparison tests were used; (** = $p < 0.01$, *** = $p < 0.001$), a statistically significant effect of all treatment groups compared with WAG/Rij (control) group. A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

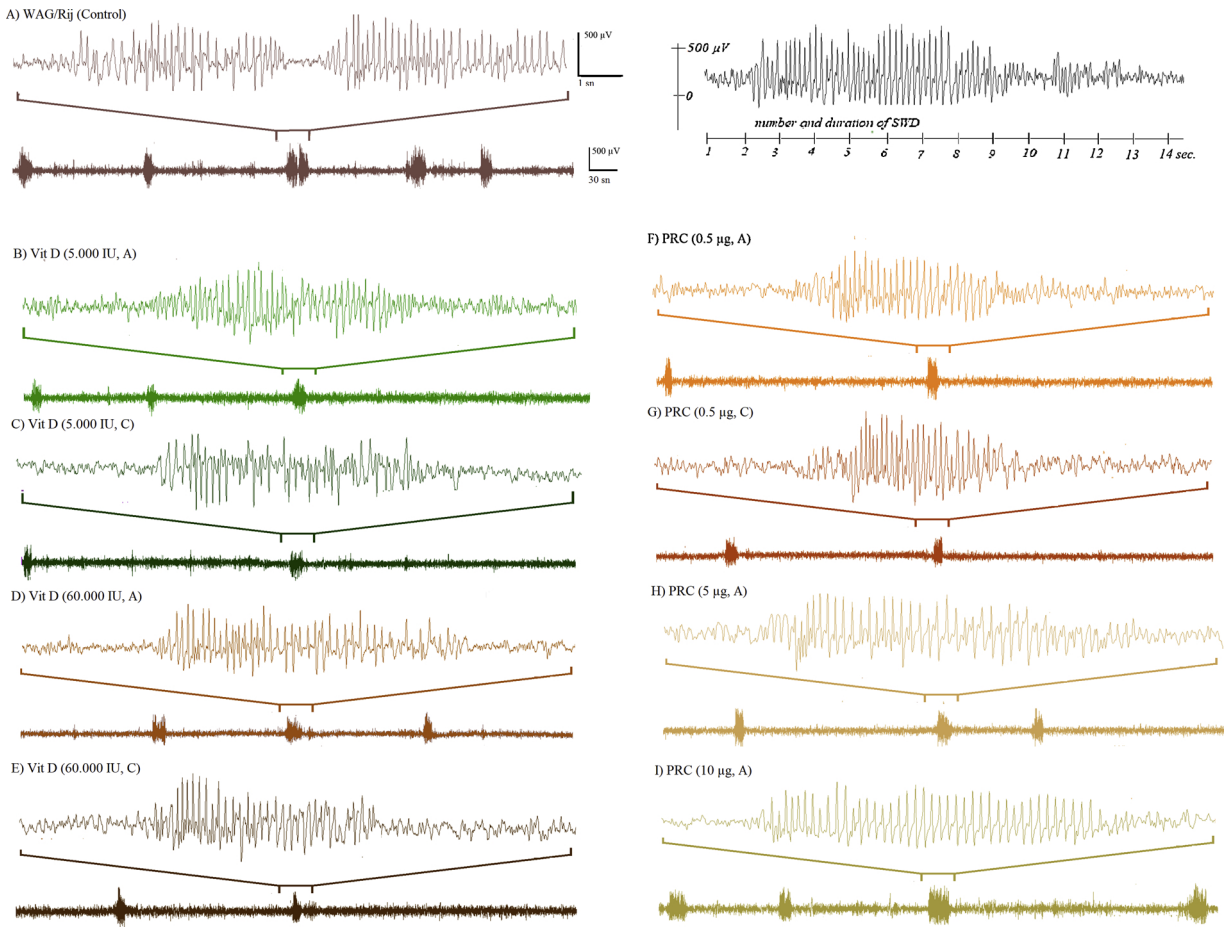

Fig. 3. Representative ECoG recordings for all groups with; (A) WAG/Rij (Control) ; (B) Vit D (5.000 IU, A); (C) Vit D (5.000 IU, C); (D) Vit D (60.000 IU, A); (E) Vit D (60.000 IU, C); (F) PRC (0.5 µg, A); (G); PRC (0.5 µg, C), (H) PRC (5 µg, A), (I) PRC (10 µg, A). Acute and chronic treatment of Vit D (5.000 IU/kg and 60.000 IU/kg) significantly decreased the number and duration of SWDs, but these parameters were more effectively reduced by chronic treatment. Acute PRC (0.5, 5 and 10 µg/kg, (A) treatment significantly decreased ECoG parameter, and a low dose of PRC (0.5 µg/kg) was the most effective dose. There were no significant changes in ECoG parameters between the acute and the chronic dose of PRC (0.5 µg/kg). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

444.0 ± 24.98 s were observed, with mean amplitudes 558 ± 20 µV within 3 h (Table 1; Figs. 3A, 4).

Acute treatment of both doses of vit D (5.000 IU/kg and 60.000 IU/kg) significantly reduced the SWD number (52.16 ± 6.22; 64%; $p < 0.01$ and 51.23 ± 5.45; 63%; $p < 0.01$, respectively) and duration (244.3 ± 28.67 s; 55%; $p < 0.001$ and 275.0 ± 22.34 s; 62%; $p < 0.001$, respectively) compared to control (Table 1; Figs. 3B,D, 4). Chronic treatment with both doses of vit D (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, single dose) significantly reduced the SWD number (41.56 ± 4.33; 51%; $p < 0.001$ and 36.67 ± 5.71; 45%; $p < 0.001$, respectively) and duration (199.3 ± 18.90 s;45%; $p < 0.001$ and 204.4 ± 34.56 s; 46%; $p < 0.001$, respectively), compared to control (Table 1; Figs. 3C, E, 4). Both doses of acute vit D treatment, similarly decreased the number and duration of SWDs, but chronic treatment was more effective in decreasing the number and duration of SWDs.

Acute treatment with different doses of PRC (0.5, 5 and 10 µg/kg) significantly reduced the duration of SWD (230.0 ± 27.46 s; 52%; $p < 0.001$, 255.5 ± 9.03 s; 57%; $p < 0.001$ and 270.2 ± 13.96 s; 60%; $p < 0.001$, respectively), compared to control (Table 1; Figs. 3F,H,I, 4). PRC (0.5 and 5 µg/kg) significantly reduced the SWD number, while PRC (10 µg/kg) did not alter SWD number, (37.5 ± 2.12; 46%; $p < 0.001$, 50.5 ± 4.01; 62%; $p < 0.001$, 67.16 ± 5.23, 82% $p > 0.05$, respectively), compared to control (Table 1; Figs. 3F, H, I, 4). PRC, at a dose of 0.5 µg /kg, was the most effective dose. Chronic PRC (0.5 µg/kg, three times per weeks, for two weeks) significantly reduced the SWD number and duration (43.20 ± 3.27; 53%; $p < 0.001$) and (239.4 ± 27.46 s; 54%; $p < 0.001$), compared to control (Table 1; Figs. 3G, 4).

Administration of vit D and PRC did not alter the amplitude of SWDs in all groups. Vit D and PRC show clear anticonvulsive properties in WAG/Rij rat.

3.2. The effects of Vitamin D and paricalcitol in anxiety-like behavior paradigms

3.2.1. Open field test

Results obtained from the open field test demonstrated that WAG/Rij rats to compared with non-epileptic Wistar rats exhibited reduced number of squares crossed (42.00 ± 3.55; 100% and 87.33 ± 5.90, 207%, $p < 0.001$, respectively), number of rearings (10.83 ± 1.50; 100% and 36.50 ± 1.75, 336%, $p < 0.001$, respectively) and duration of grooming (13.00 ± 1.21; 100% and 30.17 ± 1.38; 232% respectively). This study indicated that WAG/Rij rats exhibit anxiety-like behavior when compared with non-epileptic Wistar rats (Table 2; Fig. 5).

Acute Vit D treatment (5.000 IU/kg and 60.000 IU/kg) and PRC (0.5, 5 and 10 µg/kg), did not affect the number of rearings and duration of grooming (Fig. 4A) while significantly increasing the number of square crossing (Table 2; Fig. 5) comparing to control group (Table 1). Both chronic Vit D treatment doses (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, single dose) and effective dose of

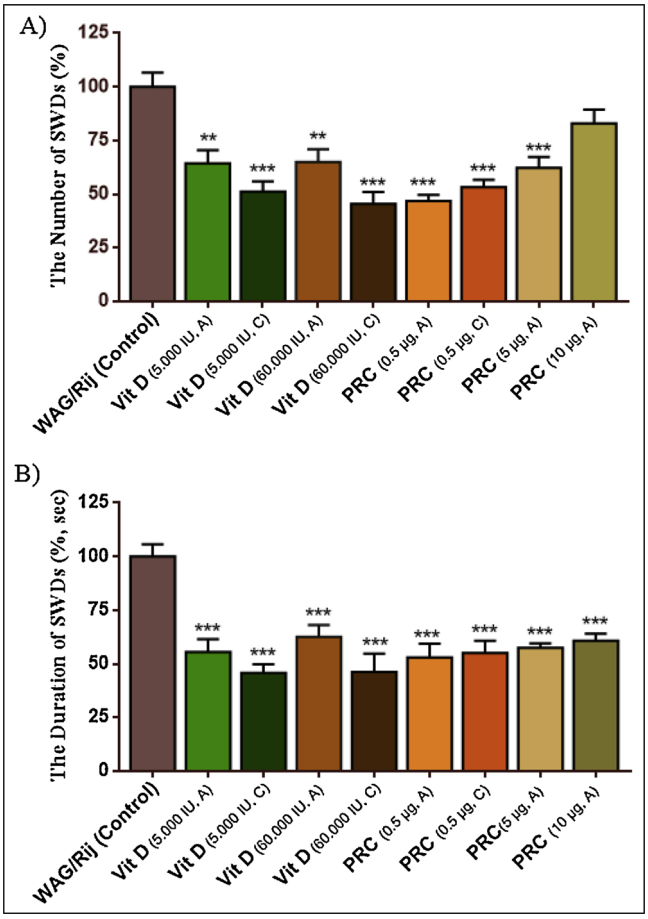

Fig. 4. Effect of acute Vit D treatment (a single doses of 5.000 IU/kg and 60.000 IU/kg) and paricalcitol (0.5, 5 and 10 µg/kg); and chronic treatment both doses of Vit D (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, single a dose) and PRC (0.5 µg/kg, 3 times per weeks, for 2 weeks) treatment on the epileptiform activity in absence-epileptic WAG/Rij rats. (A) Number of SWDs (B) and duration of SWDs.
(**=p < 0.01, ***=p < 0.001), Statistically significant effect of all treatment groups compared with WAG/Rij (control) group, (One-way ANOVA). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

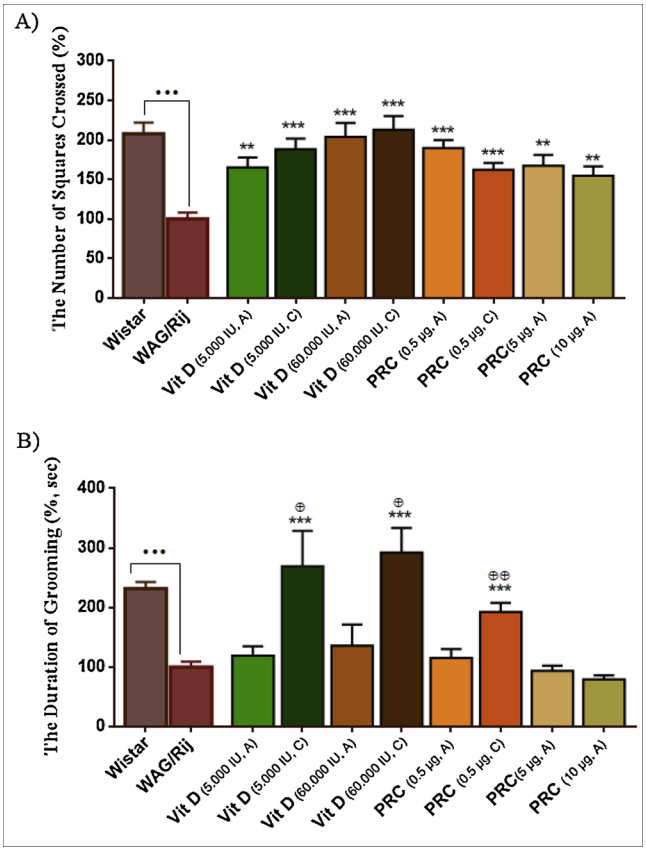

Fig. 5. Effect of acute Vit D treatment (single doses of 5.000 IU/kg and 60.000 IU/kg) and paricalcitol (0.5, 5 and 10 µg/kg); and chronic Vit D treatment doses (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, single a dose) and PRC (0.5 µg/kg, 3 times per weeks, for 2 weeks) treatment on the open field test measures in absence-epileptic WAG/Rij rats. Results are the mean ± SEM. (A) the number of square crossing (locomotor activity), (B) the duration of grooming. (••• =p < 0.001) non-epileptic Wistar rats group compared with WAG/Rij (control) rats group, (Student's *t*-test). (*= p < 0.05,**=p < 0.01, ***=p < 0.001) Statistically significance for all treatment groups compared with the WAG/Rij (control) group, (One-way ANOVA). (⊕ =p < 0.05) compared with the acute and chronic treatment group, (Paired sample *t*-test). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

PRC (0.5 µg/kg) significantly increased the number of square crossing and duration of grooming, but there was no alteration in the number of rearings comparing to control groups (Table 2; Fig. 5).

In the OFT, Vit D and PRC treatment showed some anxiolytic properties, while chronic treatment showed to be the most effective (Table 2; Fig. 5).

3.3. The effects of Vitamin D and paricalcitol in depressive-like behavior paradigms

3.3.1. Forced swimming test

Forced swim test data demonstrated that the duration of swimming time in WAG/Rij rats as compared with non-epileptic Wistar rats was

Table 2
Open field test parameters.

Groups	The Number of Square Crossing	The Duration of Grooming (sec)	The Number of Rearings
Wistar	87.33 ± 5.90	30.17 ± 1.38	36.50 ± 1.75
WAG/Rij	42.00 ± 3.55, •••	13.00 ± 1.21	10.83 ± 1.50
Vit D (5.000 IU, A)	69.33 ± 5.35,**	15.50 ± 2.06	8.83 ± 0.79
Vit D (5.000 IU, C)	79.17 ± 5.49,***	35.00 ± 7.72,***,⊕	9.50 ± 1.12
Vit D (60.000 IU, A)	85.70 ± 7.33,***	17.67 ± 4.63	11.53 ± 2.73
Vit D (60.000 IU, C)	89.50 ± 7.29,***	38.03 ± 5.29,***,⊕	12.33 ± 1.70
PRC (0.5 µg, A)	81.83 ± 4.06,***	15.00 ± 1.87	9.83 ± 1.28
PRC (0.5 µg, C)	68.17 ± 3.71,***	25.00 ± 2.29,***, ⊕ ⊕	10.00 ± 0.92
PRC (5 µg, A)	70.33 ± 5.76, **	12.23 ± 1.10	13.00 ± 0.92
PRC (10 µg, A)	65.00 ± 5.07,**	10.30 ± 0.83	11.00 ± 1.5

Data are presented as mean ± SEM. (••• =p < 0.001) non-epileptic Wistar rats group compared with WAG/Rij (control) rats group, (Student's *t*-test). (*= p < 0.05, **=p < 0.01, ***=p < 0.001) Statistically significance for all treatment groups compared with WAG/Rij (control) group, (One-way ANOVA). (⊕ =p < 0.05; ⊕ ⊕ = p < 0.01) compared with acute and chronic treatment group, (Paired sample *t*-test). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

Table 3
Forced swimming test parameters.

Groups	Immobility Time (sec.)	Swimming Time (sec.)
Wistar	136.0 ± 8.98	80.00 ± 5.83
WAG/Rij	212.8 ± 16.9 •••	42.83 ± 2.93,•••
Vit D (5.000 IU, A)	165.7 ± 13.67	62.67 ± 4.07;**
Vit D (5.000 IU, C)	149.1 ± 6.79, **	70.05 ± 5.21;***
Vit D (60.000 IU, A)	165.0 ± 13.74	65.67 ± 3.23;**
Vit D (60.000 IU, C)	141.8 ± 9.77, **	73.00 ± 4.59,***
PRC (0.5 µg, A)	173.5 ± 17.72	64.67 ± 2.89,**
PRC (0.5 µg, C)	140.8 ± 5.97, *	62.33 ± 4.42,**
PRC (5 µg, A)	176.2 ± 11.34	60.5 ± 3.40,**
PRC (10 µg, A)	180.8 ± 8.35	54.27 ± 3.55,*

Data are presented as mean ± SEM. (••• = $p < 0.001$) non-epileptic Wistar rats group compared with WAG/Rij (control) rats group, (Student's *t*-test). (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) Statistically significance for all treatment groups compared with the WAG/Rij (control) group, (One-way ANOVA). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

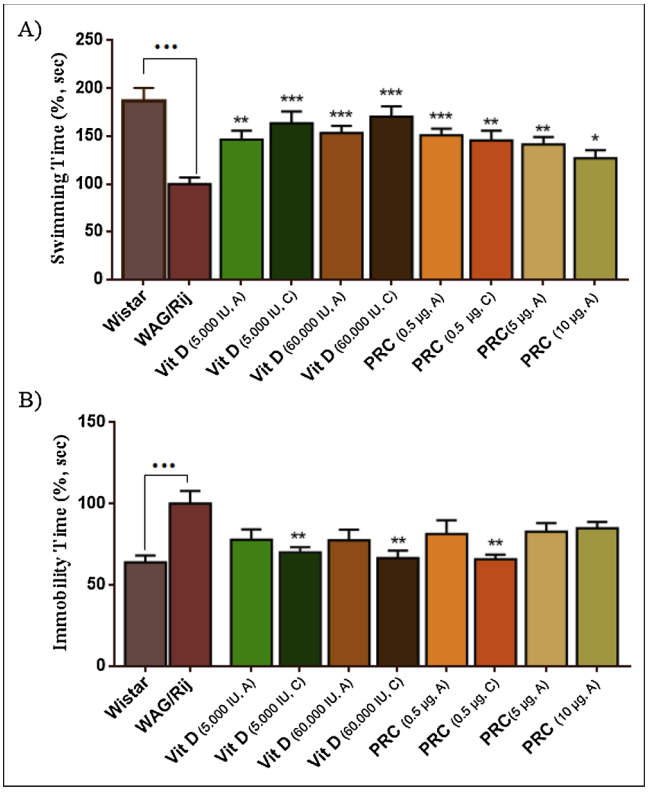

Fig. 6. Effect of acute Vit D treatment (a single doses of 5.000 IU/kg and 60.000 IU/kg) and paricalcitol (0.5, 5 and 10 µg/kg); and chronic Vit D treatment (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, a single dose) and PRC (0.5 µg/kg, 3 times per weeks, for 2 weeks) treatment on the forced swimming test measures in absence-epileptic WAG/Rij rats and non-epileptic Wistar rats (n = 7 animals per group). Results are the mean ± SEM. (A) immobility time (B) swimming time. (••• = $p < 0.001$) non-epileptic Wistar group compared with the WAG/Rij (control) group, (Student's *t*-test). (* = $p < 0.05$,** = $p < 0.01$, *** = $p < 0.001$) Statistically significance for all treatment groups compared with WAG/Rij (control) group. (One-way ANOVA). A: Acute; C: Chronic; Vit D: Vitamin D; PRC: paricalcitol.

decreased (42.83 ± 2.93 s; 100% and 80.00 ± 5.83 s; 186%, $p < 0.001$) and duration of immobility time was increased (212.8 ± 16.9 s; 100% and 136.0 ± 8.98 s; 63%, $p < 0.001$, respectively). It may be suggested that WAG/Rij rats exhibit depressive-like behavior when compared with non-epileptic Wistar rats (Table 3; Fig. 6).

Acute treatment of Vit D (5.000 IU/kg and 60.000 IU/kg) and PRC (0.5, 5 and 10 µg/kg), did not affect the duration of immobility time (Fig. 4A) while significantly increasing duration of swimming time (Table 3; Fig. 6) when compared to control groups (Table 1). Chronic treatment of both doses of Vit D (5.000 IU/kg, daily for 2 weeks and 60.000 IU/kg, bolus dose, single dose) and effective dose of PRC (0.5 µg/kg) significantly reducing the duration of immobility time and increasing duration of swimming time when compared to control groups (Table 3; Fig. 6). Chronic treatment of Vit D and PRC exhibited the most antidepressant-like effect decreasing the immobility time and increasing the swimming time in the FST when compared in WAG/Rij (control) rats.

4. Discussion

In this study, the effects of Vit D and paricalcitol on absence epilepsy in WAG/Rij rats were investigated. The results of the present study suggest that acute and chronic systemic administration of lower and highest doses of Vit D and paricalcitol showed a considerable antiepileptic and anxiolytic-antidepressive effect.

Most of the evidence has suggested that Vit D has a prominent role in the pathophysiology of epilepsy. A pilot study showed that vitamin D treatment reduced the number of seizures (40%) in epileptic patients (Hollo et al., 2012). Christiansen et al. demostrated that high doses of vitamin D supplementation significantly decreased epileptic seizures (by 30%) in patients with epilepsy. Also, many experimental animal models of seizures studies support an anticonvulsant effect for vit D (Christiansen et al., 1974). Vit D, 1.5 mg/kg/day, significantly decreased seizure frequency of epileptic episodes and increased the latency of generalized seizures in PTZ-kindled rats (Abdel-Wahab et al., 2017). Kalueff et al. (2005a) found that the doses of 50–100 µg of Vit D treatment showed an anticonvulsant effect of PTZ-induced seizures. Recently, a study showed that acute application of Vit D (2.000, 4.000 and 6.000 IU/kg i.p.) did not affect a seizure threshold, but chronically injected vitamin D3 (4.000 and 6.000 IU/kg) significantly increased seizure threshold compared to control groups (Momeni et al., 2019). 500 IU/ kg of Vit D daily for two weeks protected against hippocampal apoptosis-related with seizures induced by pentylenetetrazol and kainic acid in rats (Şahin et al., 2019). In this study, acute and chronic treatment of lower and highest doses of Vit D (5.000 IU and 60.000 IU/kg) significantly decreased the total number and duration of SWDs on ECoG activity, but there was no significant difference between the acute and chronic Vit D treatment. As for clinical usage, vitamin D is used as a bolus dose or daily dose (Priyambada et al., 2014). In the present study, there was no difference in terms of epileptiform activity between bolus dose and the daily dose of vitamin D. However, taking a single bolus dose in patients with absence epilepsy may provide convenience in terms of clinical use program. A recent clinical study conducted on patients with temporal lobe epilepsy (TLE) displayed a significant association between VDR genetic variations and the risk of TLE (Jiang et al., 2015a, 2015b). Paricalcitol, Vit D receptor agonist, at doses of 5 and 10 µg/kg showed a significant increase in first myoclonic jerk (FMJ) latencies and decreased spike percentages on PTZ-induced convulsions (Uyanıkgil et al., 2016). In this study, acute and chronic treatment of paricalcitol (0.5 µg/kg) significantly decreased the number and duration of SWDs, but there was no significant difference between acute and chronic paricalcitol treatment.

Different mechanisms have been proposed to explain the anticonvulsant effects of Vit D. Genomic and non-genomic pathways of VDR activation have been shown to play a role in vitamin D-induced neuroprotection (Garcion et al., 2002). Vitamin D's genomic actions by activated nuclear Vit D receptor and decreased expression of certain proconvulsant cytokines, such as tumor necrosis factor-alpha (TNF-a) and interleukin 1 beta (IL-1b), which increase susceptibility to seizures. In addition, Vit D can promote parvalbumin expression, which is a protein that binds calcium and prevents the release of calcium-induced neurotransmitters and protects against epileptic activity (Berridge,

2017). A previous study, systemical administration of IL-1b and TNF-a increased absence seizures in WAG/Rij rat (van Luijtelaar et al., 2012). Moreover, in another absence epilepsy model (GAERS), it was reported that IL-1b may be contributed to the onset of absence seizures (Akin et al., 2011). Increased Ca^{+2} channel expression may indirectly contribute to produces SWDs in WAG/Rij rats (van de Bovenkamp-Janssen et al., 2004). Only antiepileptogenic effects of chronic administration Vit D and paricalcitol observed in the present study might be due to the genomic actions since these genomic actions occur with a time delay of hours or days (Garcion et al., 2002). Antiepileptic effect of acute administration Vit D and paricalcitol needs to be explained by another mechanism than genomic action.

Faster Vit D motions suggesting the presence of non-genomic pathways were also identified. In the experimental epilepsy model, the anticonvulsant effect on absence epilepsy was observed 5 min after Vit D administration. (Borowicz et al., 2007). The molecular mechanisms underlying the antiepileptic activity of Vit D enhances the release of GABA in the hippocampus (Jiang et al., 2014), and this explains the non-genomic effect of Vit D. It is well known that decreasing GABAergic neurotransmission is highly relevant to the progression of epilepsy (DiNuzzo et al., 2014). However, absence epilepsy pathogenesis is related to an increased of inhibitory activity, decreased of excitatory activity and present in contrast to convulsive seizures (Tolmacheva and van Luijtelaar, 2007; Ngomba et al., 2011; D'Amore et al., 2013; Kovacs et al., 2015). Thus, if there was an increase in GABA by application of Vit D, Vit D and paricalcitol could be expected to be proconvulsant rather than anticonvulsant. The GABA-enhancing effect of Vit D does not explain the anticonvulsant effect in absence epilepsy. Unfortunately, there is no literature reporting the effects of vitamin D and paricalcitol on absence epilepsy to compare the result of the present study. As for the other mechanism, in many studies, the anticonvulsive effect of the Vit D and paricalcitol on the different experimental epilepsy animal model correlates with decreasing the inflammation and oxidative stress (Uyanıkgil et al., 2016; Liang et al., 2017). However, these theories mentioned above may not be able to explain the antiepileptic activity of paricalcitol in absence epilepsy because oxidative stress is very low in the absence epilepsy. Therefore we cannot draw a definitive conclusion from this information.

A number of studies demonstrated that the decreased locomotor activity (number of the square crossing) has been mimicked in psychomotor retardation in patients with depression (Tejani-Butt et al., 2003; Overstreet et al., 2005). Decreased number of grooming reactions in WAG/Rij rats seem to be submissive social behavior, social neglect, loss of interest or pleasure and maternal cannibalism are typically, a core symptoms of depression (Willner et al., 1992; Willner and Mitchell, 2002; Kalueff et al., 2004, 2005a, 2005b; Zou et al., 2008; Sarkisova and van Luijtelaar, 2011). Reduced number of rearings (exploratory activity) in WAG/Rij rats can represent a loss of interest in implying a deficit in novelty seeking and new situations, a symptom of anxiety for a novel environment (Sarkisova and van Luijtelaar, 2011). The increased duration of immobility time in FST seems to be the result of helplessness/hopelessness/despair, which is a characteristic feature of depressive disorder (Willner, 1990). The decreased duration of swimming time in FST seems to be the result of a failure in initiating adequate coping strategies in the stressful environment, which is also characteristic for human depression behavior (Shumake and Gonzalez-Lima, 2003).

A study showed that ovariectomized female rats treated with cholecalciferol significantly reduced the immobility time in the FST and increased the frequency of grooming in the OFT (Fedotova et al., 2016). A recent study suggested that very low doses of Vit D did not alter locomotor activity in the OFT (Sedaghat et al., 2019). Administration of 1α-Hydroxyvitamin D3 reduced immobility time but did not affect locomotor activity (Kawaura et al., 2017). The adult Vit D receptor null mutant mouse (VDR -/-) was reported to have reduced swimming activity and displayed fewer levels of grooming and rearing (Burne et al., 2006). Paricalcitol treatment decreased immobility time in lipopolysaccharide-induced depressive-like behavior rats (He et al., 2019).

This study indicated that WAG/Rij rats depressive-like behavior highlighted by a decreased swimming time and increased immobility time in the FST, also decreased the number of square crossing, the number of rearing and duration of grooming in the OFT compared to non-epileptic rats. Acute treatment of Vit D and paricalcitol increased the number of square crossing in OFT and swimming time in FST. Chronic treatment of Vit D and paricalcitol decreased immobility time in FST and increased the duration of grooming in OFT.

In the present study, no difference was found between the anticonvulsant effect of low and high dose vitamin D and low dose paricalcitol administration, whereas the anxiolytic effects of chronic administration were more potent. Studies have shown that vitamin D usage may have side effects; vomiting, stomach cramps, abdominal pain, bone loss, electrolyte imbalances, ultrafiltration loss. For this reason, paricalcitol is preferred in long-term hemodialysis patients (Teng et al., 2003). Also, it has been shown that paricalcitol administration increases serum vitamin D levels in a more controlled and subtle way. Studies have shown that the side effect of paricalcitol is considerably lower than vitamin D (Andress, 2007; Balint et al., 2000). In the lights of these results, paricalcitol may be more advantageous in patients with absence epilepsy.

5. Conclusion

This study may suggest a potential for vitamin D and paricalcitol in treating depressive and anxiety-like behavior of these animals, and they may provide significant protection against absence-epilepsy in WAG/Rij rats. There were no significant changes in ECoG and behavioural parameters in the daily and the bolus doses of Vit D. Many studies showed that seizure frequency (the number and duration of SWDs) related exacerbation in depression and anxiety-like behavior (Shaw et al., 2009). Further elucidation of these mechanisms could lead to a better understanding of the role of depression in the epileptogenesis and seizure suppression in WAG/Rij rats. In addition, antiepileptic drugs' clinical usage decrease seizure activity; yet, nearly 20–30% of epileptic patients have become intractable and poor seizure control (Weintraub et al., 2007). The clinical usage of vitamin D and paricalcitol, which are affordable drugs, might be an alternative in patients with absence epilepsy.

Author's contributions

HA: Research concept and design. HA, MA, EA: performing experiments, data collecting, analysis and interpretation of data. HA, MA, EA: Preparation of article and revisions. All authors approved the final version of the manuscript.

Acknowledgment

None.

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