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Antagonism of adenosinergic system decrease SWD occurrence via an increment in thalamic NFkB and IL-6 in absence epilepsy

Fazilet Dede[a], Sabriye Karadenizli[a], Ozgur Doga Ozsoy[b], Fatma Ceyla Eraldemir[b], Deniz Sahin[a], Nurbay Ates[a,*]

[a] Department of Physiology, Medical Faculty of Kocaeli University, Kocaeli, Turkey
[b] Department of Biochemistry, Medical Faculty of Kocaeli University, Kocaeli, Turkey

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ABSTRACT

Epilepsy is a major pathological condition, characterized by recurrent seizures and affecting approximately 1% of the population. Many studies have shown a relationship between epilepsy and inflammation. The adenosinergic system contributes to inflammation and epilepsy by regulating the release of neurotransmitters through its various receptors. This study investigates the effect of agonist and antagonist of adenosinergic system on seizure activity and cytokine levels in the WAG/Rij strain, a genetic animal model of absence epilepsy.

The WAG/Rij rats used in our study were assigned to saline, Tween 20, adenosine, and caffeine groups. Tripolar electrodes were implanted on the skull, and EEG activities recorded for 3 h. ELISA was used to determine the NFkB, TNF-α, IL-1β, and IL-6 levels in the cortical and thalamic brain regions, as well as the TNF-α, IL-1β, and IL-6 levels in the blood samples.

Administration of caffeine to rats resulted in a decreased SWD number at 30 and 60 min as determined by EEG recording after baseline ($p < .05$), and a significant increase in NFkB and IL-6 levels in the thalamic tissue ($p < .05$). Administration of adenosine to rats did not change seizures and cytokine levels.

Our results show that an increase in thalamic IL-6 and NFkB levels may related with a decrement in absence epilepsy. This study clearly shows the contribution of adenosinergic system in absence seizure in WAG/Rij rats. These results also support the importance of the thalamus on occurrence of SWD in the thalamocortical loop.

1. Introduction

Various data suggest that there is a direct relation between inflammation of the central nervous system and epileptic activity (Györffy et al., 2014). Several studies have reported that seizures are associated with the production of cytokines in the central nerveus system (CNS) (Fabene et al., 2008; Pernot et al., 2011; Silveira et al., 2012), with increased proinflammatory cytokine levels detected in several epilepsy types. For several types of epilepsy, it has been shown that increased proinflammatory cytokine levels have an effect on seizure sensitivity and brain electrical activity (Sinha et al., 2008; Shandra et al., 2002).

Absence epilepsy is a multifactorial form of absence seizure resulted from genetic and acquire factors and may contain cognitive disturbance (Franzoni et al., 2015; Pavone et al., 2001). Although several theories about the pathophysiology of absence epilepsy have been proposed, studies implicated that cortex and thalamus are involved in the generation and synchronization of spike wave discharges (SWD) (Matricardi et al., 2014; Meeren et al., 2002). Spontaneously occuring SWD activity are consider to a distinguishing marker of epileptic strain. WAG/Rij (Wistar Albino Glaxo/Rijswijk) is an approved genetic animal model of absence epilepsy, showing SWDs with duration 1–30 s and a frequency of 7–10 Hz. While SWDs do not become present in EEG recording before 2–3 months of age, they clearly emerge in 6-month-old rats. Generally, absence seizures are accompanied by myoclonic jerks of the face, eye twitching, and respiratory acceleration. SWDs are affected by wakefulness situations such as awaking, slow-wave sleep, and rapid eye movement (Coenen and Van Luijtelaar, 2003).

Adenosine is a purine nucleoside produced during metabolic stress conditions such as ischemia, inflammation, and tissue injury (Safarzadeh et al., 2016). It plays a major role in the regulation of immune homeostasis (Olah and Stiles, 1995). Adenosine has

Abbreviations: CNS, Central nervous system; SWD, spike wave discharge; EEG, electroencephalogram; WAG/Rij, Wistar Albino Glaxo/Rijswijk; NFkB, *nuclear factor kappa B*

* Corresponding author at: Department of Physiology, Medical Faculty of Kocaeli University, 41380 Umuttepe, Kocaeli, Turkey.
E-mail address: nates@kocaeli.edu.tr (N. Ates).

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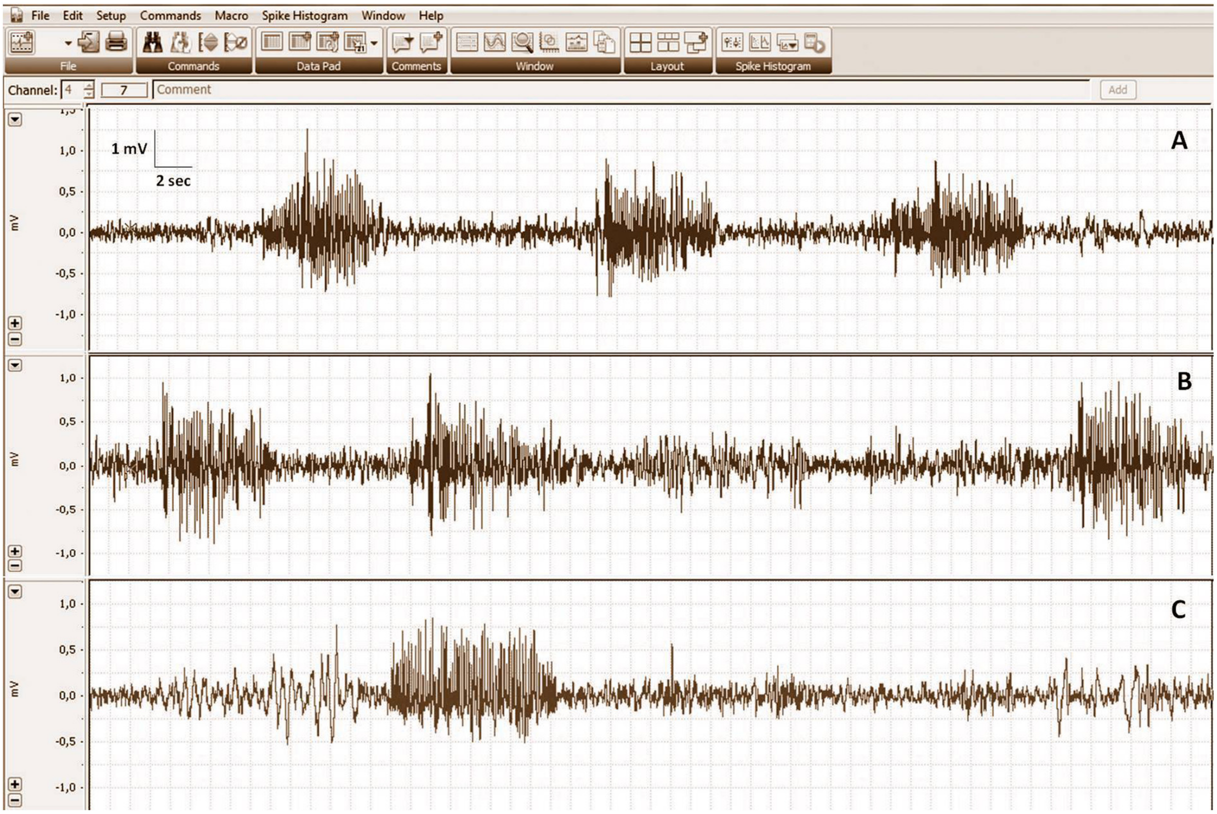

Fig. 1. SWD in EEG recording of 6–8 months WAG/Rij rats (two edges' length is 1 milivolt (mV) in the vertical plane, two edge length is 2 s in the horizontal plane). (A) Salin control group EEG recording; (B) Adenosine group EEG recording; (C) Caffeine group EEG recording. This EEG was recorded in the first hour after injection.

Table 1
The effects of adenosinergic system modulation on the number of SWDs in absence epilepsy. Results are represented as mean ± SEM. After drug injection, EEG data were separated into 4 epochs of 30, 60, 90, and 120 min.

Groups	*n*	30 min	60 min	90 min	120 min
Saline	7	30.5 ± 5.37	20 ± 4.03	17.5 ± 4.63	17.83 ± 4.54
Tween 20	7	17.12 ± 7.76	11.5 ± 4.75	4.37 ± 1.82	5.62 ± 1.25
Caffeine	7	3.57 ± 1.49**	6.28 ± 1.5	7 ± 1.97	8.85 ± 2.93
Adenosine	7	19.55 ± 6.07*	23.66 ± 6.12*	13 ± 3.27	13.55 ± 3.21

** $p < .001$ vs. saline group.
* $p < .05$ vs. caffeine group.

anticonvulsant and neuroprotective properties as an endogen modulator (Boison, 2012). Caffeine (1,3,7-trimethylxanthine), which is structurally similar to adenosine, is a nonspecific adenosine receptor antagonist. Caffeine may reduce neuroinflammation by regulating the adenosine receptor-mediated modulation of glutamate release. It has been shown that while chronic caffeine consumption provides neuronal protection against epileptic damage in rats, acute high-dose caffeine has an adverse effect in the animal models of epilepsy (Souza et al., 2013).

In this study, we investigated the effect of adenosinergic modulation on the levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6), NFkB, and seizure activity in genetic absence epileptic WAG/Rij rats.

2. Material and methods

2.1. Animals

WAG/Rij male rats (n = 28; 6–8 months old, 289 ± 27.35 g) were obtained from the Kocaeli University Experimental Medicine Research and Implementation Unit. The animals were housed in a temperature-controlled (25 °C) room and maintained on a 12:12 h light-dark cycle. The animals had ad libitum access to pelleted chow and water. The experimental design was approved by the Ethics Committee for Animal Care and Use of the Medical Faculty of Kocaeli University (Protocol No. 4/9-2016).

The animals were randomly divided into 4 groups and injected as per the following protocol.

1. Group: Saline Group n:7 (i.p. saline injection).
2. Group: Tween 20 Group n:7 (i.p. 8% Tween 20 injection).
3. Group: Caffeine Group n:7 (5 mg/kg/i.p.) (Germé et al., 2015).
4. Group: Adenosine Group n:7 (120 mg/kg /i.p.) (Ilbay et al., 2001).

Table 2
The effects of adenosinergic system modulation on total SWD duration in absence epilepsy. Results are represented as mean ± SEM. After drug injection, EEG data were separated into 4 epochs of 30, 60, 90, and 120 min.

Groups	*n*	30 min	60 min	90 min	120 min
Saline	7	116.92 ± 7.97	63.38 ± 15.67	56.64 ± 14.72	74.72 ± 16.53
Tween 20	7	112.83 ± 50.29	66.57 ± 24.02	30.46 ± 12.68	34.32 ± 10.13
Caffeine	7	29.6 ± 13.03	35.7 ± 10.37	36.06 ± 9.06	52.54 ± 18.43
Adenosine	7	153.90 ± 58.51*	146.36 ± 41.48*	95.44 ± 27.93	89.38 ± 25.87

* $p < .05$ vs. caffeine group.

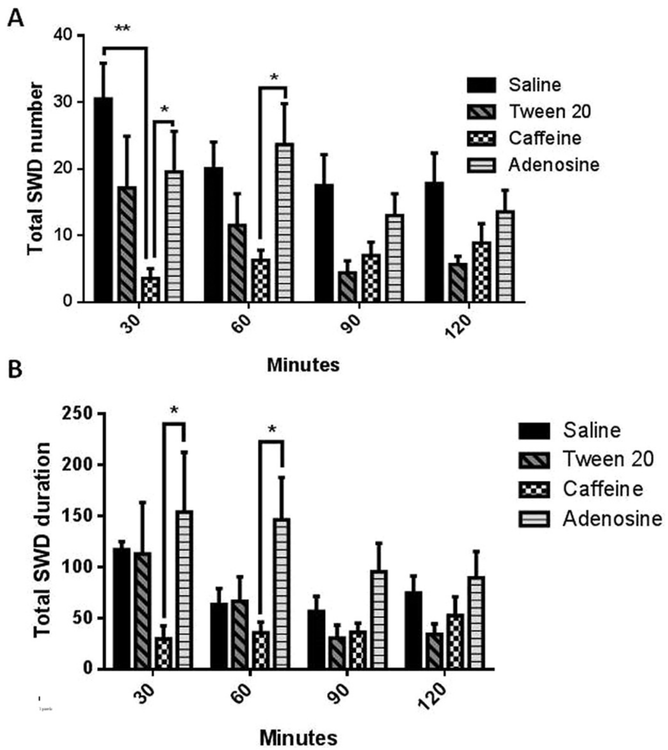

Fig. 2. The effects of adenosinergic system modulation on the number of SWDs and total SWD duration in absence epilepsy. After drug injection, EEG data were separated into 4 epochs of 30, 60, 90, and 120 min. Bars represent mean ± SEM. A. Total SWD number and $^{**}p < .001$ vs. Saline group; $^{*}p < .05$ vs. caffeine group. B. Total SWD duration and $^{*}p < .05$ vs. caffeine groups.

Table 3
The effects of adenosinergic system modulation on NFkB, TNF-α, IL-1β, and IL-6 levels in the cortex of WAG/Rij rats. Results are represented as mean ± SEM.

Groups	Cortex NFkB, pg/mgprotein	Cortex TNF-α, pg/mgprotein	Cortex IL-1β, pg/mgprotein	Cortex IL-6, pg/mgprotein
Saline	732.8 ± 97.73	59.63 ± 13.48	530.6 ± 25.83	1348 ± 233.1
Tween 20	656 ± 92.1	53.81 ± 10.64	598.8 ± 81.04	1321 ± 196.5
Caffeine	813.6 ± 62.5	62.92 ± 8.5	670.8 ± 29.33	1504 ± 92.7
Adenosine	949.9 ± 117.9	63.68 ± 10.81	633.5 ± 60.75	1394 ± 175.1

2.2. Drugs

Adenosine (A9251) and caffeine (C0750) were purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). Caffeine was dissolved in saline; adenosine was dissolved in 8% Tween 20. All drugs were administered by intraperitoneal/i.p. injection.

2.3. Electrode implantation

Tripolar electrodes (Plastic Products Company, MS 333/2A) were implanted under complete ketamine (90 mg/kg, i.p.) / xylazine (12 mg/kg, i.p.) anesthesia. The bregma coordinates were: A 2.0, L 3.5; A –6.0, L 4.0 (respectively, frontal cortex and occipital cortex), according to a previously described protocol (Sahin et al., 2009), and a reference electrode was placed on the cerebellum. Then the electrodes were fixed by placing two screws in the skull using cold dentacrylate cement. Rats were allowed a week for recovery.

After recovery, the rats were habituated to the EEG recording system one day before the experiment. On the experiment day, after the 1 h baseline period (between 9 am and 10 am), drugs were injected and EEGs recorded for 2 h (between 10 am and 12 am). Electrophysiological data analysis was done using the software package LabChart7.

2.4. Collecting blood and tissue samples

After EEG recording, intracardiac blood samples were collected from the rats under ketamine and xylazine anesthesia. Blood samples were centrifuged and supernatants collected. Then the brains were removed, and cerebral cortex and thalamus tissues isolated in a cold environment. Tissue samples were stored at −40 °C until analysis.

2.5. Tissue homogenization

The thalamus and cortex tissues were homogenized with a tissue homogenizer in phosphate-buffered saline (1/10; weight/volume) (Calkins et al., 2001). After homogenization, the samples were centrifuged for 20 min at 3000 rpm and the supernatant collected for analysis.

2.6. Determination of total protein in tissue samples

The total protein content of the tissue samples was quantified by the modified Lowry method (Hartree, 1972). ELISA results were given as a percentage of the total protein concentration of the sample.

2.7. Measurement of NFkB, TNF-α, IL-1β, and IL-6 with ELISA

NFkB concentration in the cortex and thalamus, and TNF-α, IL-1β, and IL-6 concentrations in the cortex, thalamus, and serum, were determined using commercial ELISA kits (eBioscience; Vienna, Austria) following the manufacturer's instructions.

2.8. Statistical analysis

The statistical analyses were performed using the GraphPad Prism package. All data were expressed as mean ± SEM. The Kolmogorov-Smirnov test was used to assess the normality of the data. To compare multiple groups, we used a Two-Way ANOVA with the post-hoc Tukey test for seizure parameters or a one-way analysis of variance (ANOVA) with the post-hoc Tukey test for other parameters. $p < .05$ was considered statistically significant.

3. Results

3.1. Evaluation of spike-wave discharge in absence seizures

SWD activity was observed in EEG recordings of all the WAG/Rij. We determined SWD activity by reference to patterns that were at least twice the size of the baseline EEG line and lasted > 2 s (Fig.1).

3.2. The effect of adenosine and caffeine on the number of SWDs and total SWD duration

All animals were acclimatized to the EEG recording instrument a day before the experiment. One the experiment day, after the 1 h baseline period, drugs were injected and EEGs recorded for 2 h. After all recordings, we analyzed the baseline period and drug period as regards total duration and number of SWDs. Then the drug period was separated into 4 epochs of 30 min (Tables 1 and 2).

We did not find statistically significant results between the groups for the total duration of SWD and the number of SWDs in the baseline EEG ($p > .05$). Further, the number of SWDs in the Tween 20 control group did not change significantly during the 3 h compared to the saline group ($p > .05$). After the first 30 min from the caffeine treatment, we observed a significant decrement in the number of SWDs compared to the saline group ($p < .001$) and the adenosine group ($p < .05$). But we

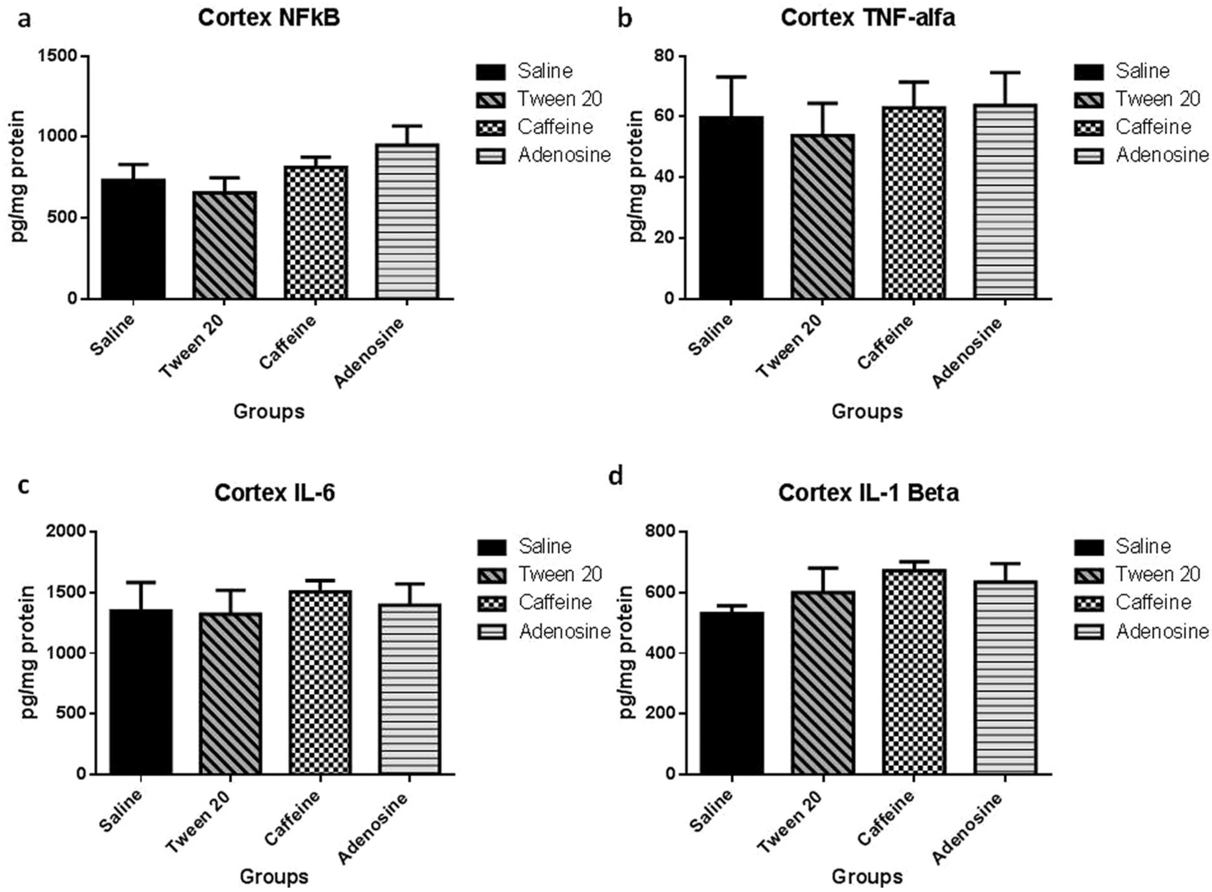

Fig. 3. Effects of adenosinergic modulation on NFkB, TNF-α, IL-1β, and IL-6 levels in the cortex of WAG/Rij rats. a Cortex NFkB. b Cortex TNF-α. c Cortex IL-1β. d Cortex IL-6. Bars represent mean ± SEM. n = 7.

Table 4
The effects of adenosinergic system modulation on NFkB, TNF-α, IL-1β, and IL-6 levels in the thalamus of WAG/Rij rats. Results are represented as mean ± SEM.

Groups	Thalamus NFkB, pg/mgprotein	Thalamus TNF-α, pg/mgprotein	Thalamus IL-1β, pg/mgprotein	Thalamus IL-6, pg/mgprotein
Saline	449.4 ± 57.32	32.63 ± 8.26	427.7 ± 83.12	842.4 ± 167.9
Tween20	638.1 ± 76.39	44.72 ± 6.49	543.7 ± 82.18	1087 ± 160.8
Caffeine	886.3 ± 89.42**	69.05 ± 15.69	610.7 ± 58.85	2133 ± 418.5*
Adenosine	647.7 ± 89.23	59.25 ± 11.42	627.5 ± 63.76	1526 ± 233

n = 7.
* $p < .05$, vs. saline and Tween 20 groups.
** $p < .05$, vs. saline group.

did not find statistically significant results between the adenosine group and the saline group ($p > .05$). Sixty minutes after the caffeine treatment, the number of SWDs had decreased compared to the adenosine group ($p < .05$). We did not obtain significant results between the groups in the other epochs (90 and 120) as regards both total SWD number and total SWD duration (Table 1 and Fig. 1). Furthermore, 30 and 60 min from the caffeine treatment we observed a significant decrement in total SWD duration compared to the adenosine group ($p < .05$) (Table 2 and Fig.2).

3.3. Evaluation of inflammatory cytokine and NFkB levels in the samples

The levels of NFkB, TNF-α, IL-1β, and IL-6 in the cortex are presented in Table 3. NFkB, TNF-α, IL-1β, and IL-6 levels in the cortex did not exhibit significant results between groups ($p > .05$; Fig. 2). Cortex NFkB results increased slightly but not significantly in the caffeine and adenosine groups compared to the saline group. Cortex IL-6 and IL-1β levels increased slightly but not significantly in the caffeine group compared to the saline group ($p > .05$). (Table 3, Fig. 3).

The levels of NFkB, TNF-α, IL-1β, and IL-6 in the thalamus are presented in Table 4.

Thalamus NFkB results increased significantly in the caffeine group compared to the saline group ($p < .05$). Further, thalamus IL-6 results increased significantly in the caffeine group compared to the saline and Tween 20 groups ($p < .05$). Thalamus NFkB and IL-6 levels increased slightly but not significantly in the adenosine group compared to the saline control group ($p > .05$). Thalamus TNF-α and IL-1β levels increased slightly but not significantly in the caffeine and adenosine groups compared to the saline control group (Fig.4).

The levels of TNF-α, IL-1β, and IL-6 in the serum are presented in Table 5. Serum TNF-α levels increased significantly in the caffeine group compared to the saline group ($p < .001$) and adenosine group ($p < .05$). Serum IL-1β levels increased significantly in the caffeine group compared to the adenosine group ($p < .05$). Serum IL-6 levels increased slightly but not significantly in the caffeine compared to the saline control group ($p > .05$) (Fig. 5). The results indicated that

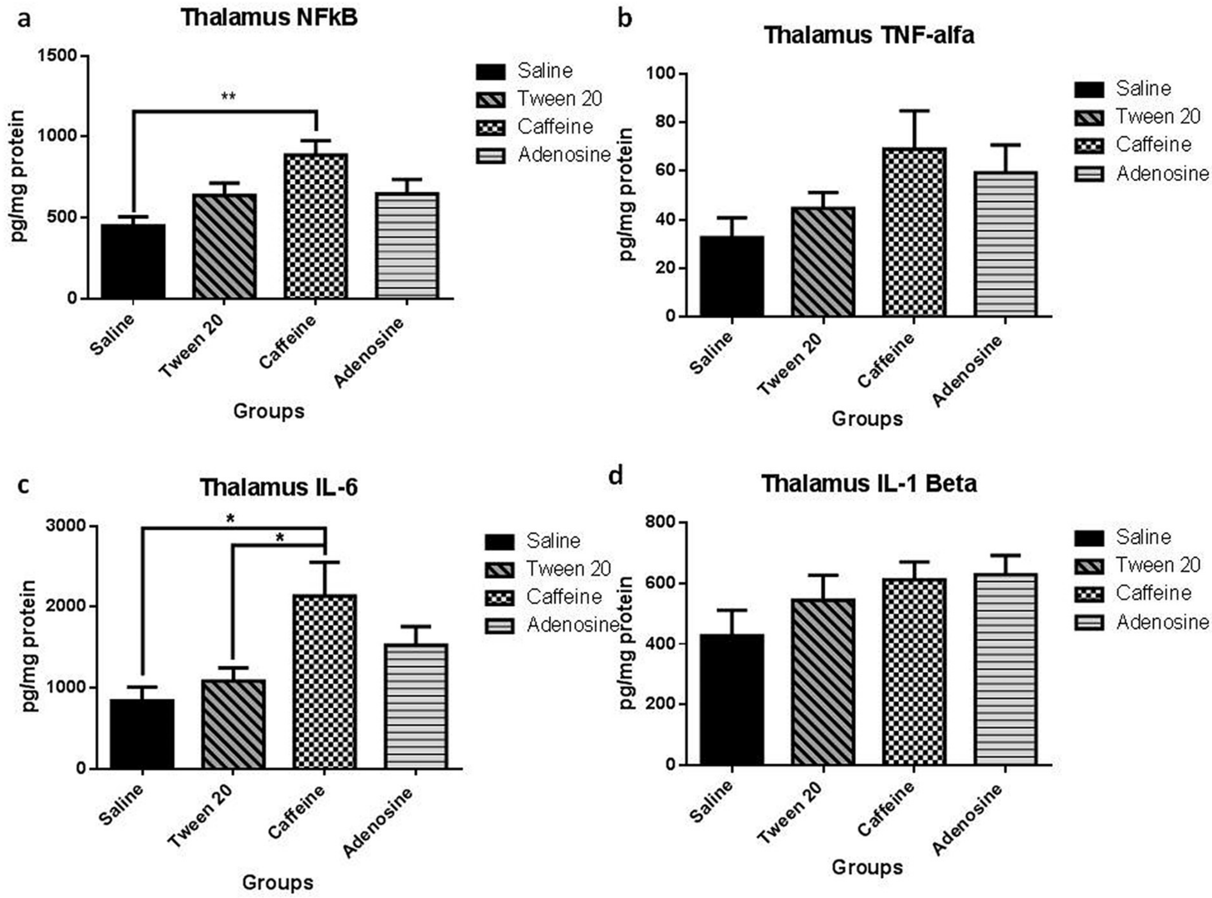

Fig. 4. Effects of adenosinergic modulation on NFkB, TNF-α, IL-1β, and IL-6 levels of thalamus in WAG/Rij rats. a Thalamus NFkB. b Thalamus TNF-α. c Thalamus IL-1β. d Thalamus IL-6. Bars represent mean ± SEM. $^{*}p < .05$, vs saline and Tween 20 groups. $^{**}p < .05$, vs saline group. $n = 7$.

Table 5
The effects of adenosinergic system modulation on TNF-α, IL-1β, and IL-6 levels in the serum of WAG/Rij rats. Results are represented as mean ± SEM.

Groups	Serum TNF-α, pg/ml	Serum IL-1β, pg/ml	Serum IL-6, pg/ml
Saline	44.59 ± 9.9	1067 ± 176.1	2873 ± 195.8
Tween20	61.89 ± 10.95	771.5 ± 96.54	2445 ± 262.7
Caffeine	165.8 ± 28.51**	1484 ± 172*	3599 ± 424
Adenosine	54.31 ± 6.43	855.9 ± 147.4	2614 ± 299.9

** $p < .001$ vs saline group.
* $p < .05$ vs adenosine group.

caffeine administration increased the level of inflammatory molecules in systemic circulation.

4. Discussion

In this study, we investigated the effect of adenosinergic system modulation on NFkB, cytokine levels, and seizure activity in genetic absence epileptic WAG/Rij rats. Caffeine treatment decreased the number of SWD at 30 and 60 min, but increased IL-6 and NFkB levels in thalamus tissue and IL-1β and TNF-α levels in serum. These results show that low-dose acute caffeine administration has an effect on absence epilepsy through a proinflammatory way in the systemic circulation and an anti-inflammatory way in the thalamic tissue.

Many studies have indicated that chronic caffeine administration, but not acute caffeine administration, has an effect on convulsive seizure (Souza et al., 2013; Dede et al., 2017). It has been shown that low-dose acute caffeine administration can decrease the number of SWDs because of the antagonistic effect of caffeine on the adenosine A1 and A2 receptor in the GAERS rat strain (Germé et al., 2015). These findings support our results, which indicate that caffeine decreases absence seizure activity.

Caffeine blocks the A2A receptor, which has been reported as leading to a reduction in Na + influx through a decrease in glutamate release. Also, it has been found that caffeine inhibits neurotransmitter release and depolarization by opening the K+ channel, and inhibits excitotoxicity by altering the neuronal metabolism (Mao et al., 2007; Xu et al., 2005). These studies have supported that caffeine causes a decrement in seizures by blocking the A2A receptor, and the results are in line with our study. It is known that sleep deprivation causes an increase of SWDs in WAG/Rij, and also that caffeine consumption restores the inflammatory changes throughout sleep deprivation (Drinkenburg et al., 1995; Wadhwa et al., 2018). The effect of caffeine on sleep deprivation may be contributing to the decreasing SWD number in our study.

Adenosine pretreatment has been shown to prolong the latency of generalized seizures and also to decrease cytokine concentrations in the thalamus and the cortex (Dede et al., 2017). It has been reported that thalamocortical excitation is regulated by presynaptic A1 receptors, and that cortical excitation could be decreased by an increase in adenosine levels (Fontanez and Porter, 2006). Another study has shown that administration of adenosine increased epileptic seizure frequency in WAG/Rij (Ilbay et al., 2001). According to these studies, adenosinergic modulation has the opposite effect on convulsive and non-convulsive seizures (Germé et al., 2015; Dede et al., 2017; Ilbay et al., 2001). It has been reported that adenosine activity was decreased the neuronal firing rate in the cerebral cortex/thalamus of WAG/Rij and Long Evans rats. This activation may be resulting from inhibition predominantly of the adenosine A1 receptor in the neocortex and thalamus (Kovács et al.,

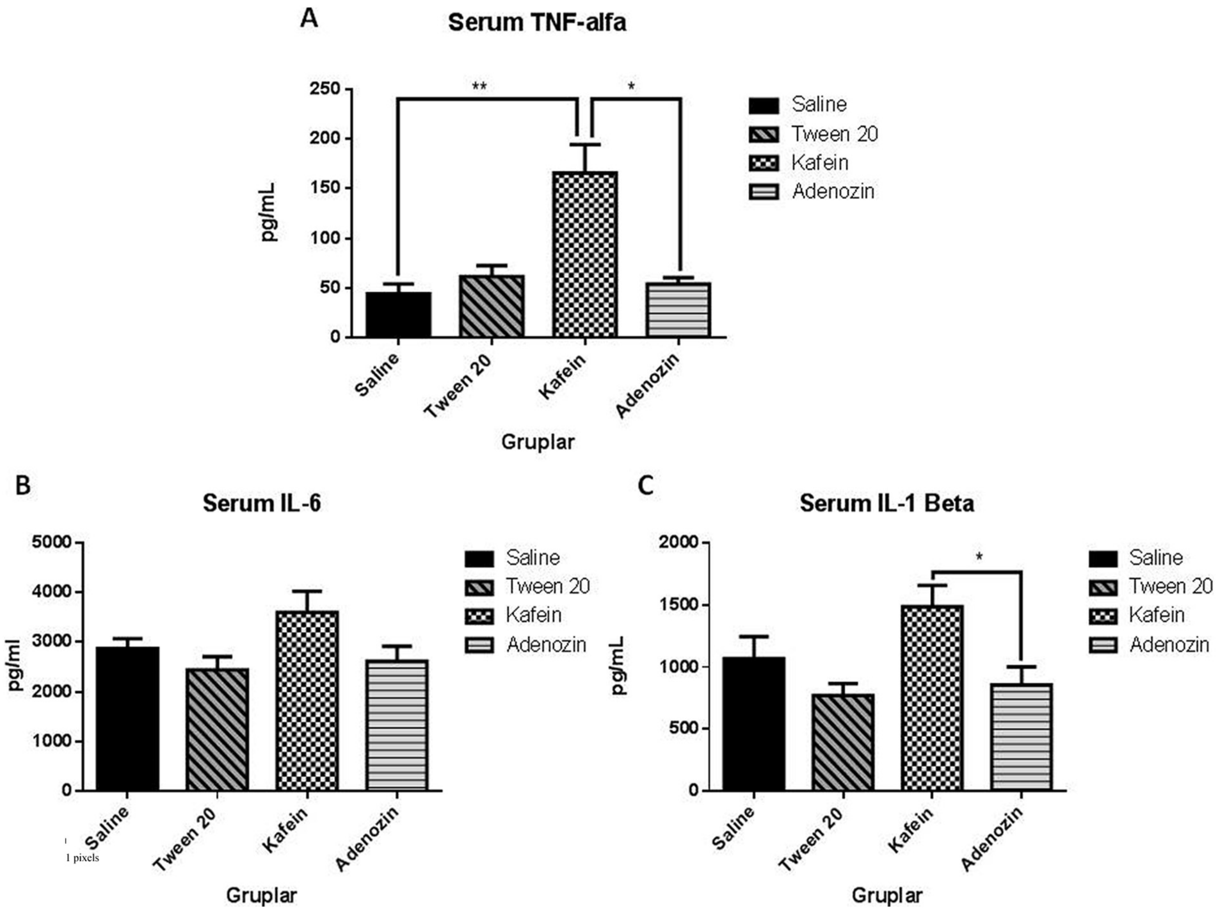

Fig. 5. Effects of adenosinergic modulation on TNF-α, IL-1β, and IL-6 levels in the serum of WAG/Rij rats. a Serum TNF-α. b Serum IL-6. c Serum IL-1β. Bars represent mean ± SEM. $^{**}p < .001$ vs saline group; $^{*}p < .05$ vs adenosine group.

2013). The results of our study are in line with these findings. Our study showed that adenosine usage causes a tendency to decrease both SWD number and total SWD duration, but did not find a statistically significant.

Cytokines are important molecules that mediate cell-to-cell communication through the immune response and inflammation (Xing et al., 1998). The importance of the balance between anti-inflammatory and pro-inflammatory cytokines is well known in the development of epilepsy (Rana and Musto, 2018). Also, it has been found that TNF-α, IL-1β, and IL-6 levels are elevated in the brain, serum, and cerebrospinal fluid of the different seizure models (Dede et al., 2017; Rao et al., 2009). IL-6 plays an important role as the anti-inflammatory agent in acute and systemic inflammation by controlling proinflammatory cytokine levels (Xing et al., 1998). IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties (Scheller et al., 2011). Our study shows a significant increase of IL-6 levels in thalamus tissue after administration of caffeine, but not in the cortex and serum. We conclude in this study that IL-6 has anti-inflammatory properties. The reason for the increasing IL-6 levels in the thalamus may be the anti-inflammatory properties of caffeine. Many studies have shown that caffeine has anti-inflammatory properties, and contributes to the inhibition of TNF- α expression by decreasing the number of activated microglia (Hosny et al., 2018; Brothers et al., 2010; Kang et al., 2012). Also, the increase in extracellular adenosine induces IL-6 expression by the A2B receptor, and induction of IL-6 expression depends on the transcriptional effect of NFkB (Schwaninger et al., 1997). Our results are consistent with these studies. The results obtained show that caffeine may reduce the number of SWDs because of increases in thalamic IL-6 and NFkB. These results reveal the importance of the thalamus in the thalamocortical loop of absence epilepsy.

It is known that IL-1β increases absence seizures, and TNF-α delays the effect of SWD, but it remains unknown whether IL-6 modulates the occurrence of SWDs (van Luijtelaar et al., 2012). Our study suggests that an increase in thalamic IL-6 levels reduces the number of SWDs. A2B adenosine receptor activation elevates IL-6 mRNA and protein levels through the PLC, PKC-ε, PKC-δ, and p38 pathways in a primary microglia culture (Merighi et al., 2017). Although microglia has all the adenosine receptor subtypes, the A2B receptor is responsible for the increase in IL-6 levels (Merighi et al., 2017). In our study, caffeine, a non-selective adenosine receptor antagonist, may lead to increasing thalamic IL-6 levels by having a stimulatory effect on the A2B receptor.

The activation signal of IL-1β increases limbic seizures in the forebrain of adult rats (Balosso et al., 2008; Vezzani et al., 2008), and contributes to the occurrence of febrile-like convulsions in immature rats (Dubé et al., 2005). Another study has reported that activated astrocytes by IL-1β stimulation in the somatosensory cortex of GAERS contributed to the process of SWD maturation. Moreover, it has also been reported that blocking of IL-1β biosynthesis is an anti-inflammatory approach which may contribute to SWD activity (Akin et al., 2011). We observed that IL-1β and TNF-α in thalamic and cortical tissue are not significantly affected by adenosinergic modulation, but that there is a significant increase of TNF-α in the blood samples of caffeine group. van Luijtelaar et al. (2012) reported that intraperitoneal TNF-α injection did not have a direct effect on SWD in WAG/Rij, and that other physiological and hormonal mechanisms may be contributing to the late effect of TNF-α. The results of this study support that findings. It has been reported that TNF-α is revealed by IL-1β induced in absence epilepsy (van Luijtelaar et al., 2012). When we observe systemic cytokine levels, IL-1β may be induced to TNF-α through the effect of caffeine on IL-1β and TNF-α. Also, there is an opposite relationship between TNF-α and SWD which decreases age-dependent serum TNF-α levels contrary to the increase in the number of SWD (van Luijtelaar et al., 2012). The differences in function of macrophages have been indicated rather than a uniform activity. Thus, macrophages has been classified as M1 and M2 to show functionaly distinct state of macrophage. While M1 macrophages are characterized with inflammatory secretion and production of nitric oxide and, activation induced by intracellular pathogens, lipoprotein, cytokine such as INFγ and TNFα, M2 macrophages have complex role outside the context of inflammation such as; organ morphogenesis, tissue turnover, endocrine signaling and, activation induced by immune complex complement, IL-1, IL-10, TGFβ (Murray et al., 2014; Röszer, 2015). Under the light of this data, it can be said that the observed effect of IL-6 might be took placed probably via M2 pathway.

Furthermore, causes of differences in statistically significant cytokine levels in the cortex and thalamus may arise from local properties of the brain. It has been shown that the neurons of the neocortex, hippocampus, basal ganglion, and thalamus have strong cytokine immunoreactivity in the periventricular leukomalacia brain, as well as different cytokine levels locally (Kadhim et al., 2003). Also, it has been shown that cytokine levels increase in different concentrations in different brain areas in kainic acid-induced seizures (Lehtimäki et al., 2003). Various studies have pointed out that these differences were not revealed accidentally (Kadhim et al., 2003; Lehtimäki et al., 2003). Our study shows that cytokine concentrations differ between the thalamus and cortex, and this situation may be important for absence epilepsy.

In conclusion, this study has clearly demonstrated the effects of adenosinergic modulation and related cytokines on absence epilepsy. An increase in thalamic IL-6 and NFkB levels and a reduction in the number of SWDs after caffeine administration was the most apparent finding. Consequently, NFkB and IL-6 participate in the pathogenesis of absence seizures, and the activation of NFkB and IL-6 with adenosinergic modulation may decrease absence seizures.

The limitation of the studies is about the sample size due to ethical reasons.

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