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ARAŞTIRMA / RESEARCH

Cardiovascular changes in WAG/Rij rats with genetic absence epilepsy: Effects of chronic ethosuximide treatment

Genetik absans epilepsili WAG/Rij sıçanlarda kardiyovasküler değişiklikler: Kronik etosüksimid tedavisinin etkileri

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Abstract

Purpose: The aim of this study was to investigate the effects of chronic ethosuximide (ETX) treatment on absence seizures and cardiovascular parameters in WAG/Rij rats with genetic absence epilepsy.
Materials and Methods: Eight-weeks old, male Wistar and WAG/Rij rats were divided into four groups (n=20): Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX. ETX groups received chronic ETX treatment (oral, 300 mg/kg/day) for 3 months. At the end of the 3-month-treatment period; the total and mean duration, also number of spike wave discharges (SWDs) were evaluated using EEG recordings. Mean arterial blood pressure (MAP) and heart rate (HR) measurements were performed.
Results: ETX treatment significantly decreased the duration and frequency of SWDs in WAG/Rij rats. MAP in WAG/Rij control group was markedly higher than Wistar control group. In Wistar ETX group, HR was significantly slower than Wistar control group. KCl-induced contraction response enhanced in Wistar ETX group and diminished in WAG/Rij control group compared to Wistar control group.
Conclusion: Increased MAP and vascular reactivity in WAG/Rij rats. ETX treatment did not alter cardiovascular parameters in WAG/Rij rats whereas the treatment decreased the HR and vascular reactivity without affecting MAP in Wistar rats. T-type Ca^{++} channels may play a role in these changes.

Keywords:. Absence epilepsy, WAG/Rij, ethosuximide, heart rate, blood pressure, vascular reactivity

Öz

Amaç: Bu çalışmada, genetik absans epilepsili WAG/Rij sıçanlarda kronik etosüksimid (ETS) tedavisinin absans nöbetler ve gözlenebilecek olası kardiyovasküler değişiklikler üzerindeki etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: İki aylık erkek Wistar ve WAG/Rij sıçanlar; Wistar kontrol, Wistar ETS, WAG/Rij kontrol ve WAG/Rij ETS gruplarına ayrılmıştır (n=20). ETS grupları 3 ay boyunca kronik ETS tedavisi (oral, 300 mg/kg/gün) almışlardır. Üç aylık tedavi periyodunun sonunda EEG kayıtları kullanılarak diken dalga deşarjlarının (DDD) toplam süre, ortalama süre ve sayısı değerlendirilmiştir. Ortalama arteriyel kan basıncı (OAB) ve kalp hızı (KH) ölçümleri gerçekleştirilmiştir.
Bulgular: ETS tedavisi WAG/Rij sıçanlarda DDD'leri toplam süre, ortalama süre ve sayı açısından anlamlı olarak azaltmıştır. OAB, WAG/Rij kontrol grubunda Wistar kontrol grubuna göre anlamlı olarak artmıştır. KH, Wistar ETS grubunda Wistar kontrol grubuna göre anlamlı olarak azalmıştır. KCl aracılı kasılma yanıtlarında Wistar kontrol grubuna göre Wistar ETS grubunda anlamlı artma, WAG/Rij kontrol grubunda anlamlı azalma saptanmıştır.
Sonuç: Sonuçlarımız OAB ve vasküler reaktivitenin WAG/Rij sıçanlarda arttığını göstermiştir. ETS tedavisi, WAG/Rij sıçanlarda kardiyovasküler parametreleri değiştirmezken Wistar sıçanlarda KH ve vasküler reaktiviteyi azaltmış OAB'yi etkilememiştir. Bu değişikliklerde T-tipi Ca^{++} kanallarının rol oynayabileceği düşünülmektedir.

Anahtar kelimeler: Absans epilepsi, WAG/Rij, etosüksimid, kalp hızı, kan basıncı, vasküler reaktivite

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INTRODUCTION

It is known that seizures in patients with epilepsy affect heart rate and rhythm by causing autonomic neuronal dysfunction, and recurrent seizures may result in severe clinical consequences by impairing cardiac function[1]. Cardiac arrhythmias, one of the possible causes of sudden unexpected deaths in epilepsy, led to the investigation of cardiovascular functions in epilepsy[2]. Although there have been researches focused on cardiovascular functions in many types of epilepsy, studies exploring cardiovascular functions in patients with absence epilepsy or in absence epileptic animals are limited.

Absence epilepsy, generally develops in childhood, is a neurological condition characterized by spike wave discharges (SWDs) occurring in EEG, which reflects spontaneous recurrent seizures[3]. WAG/Rij and GAERS rats are valid genetic animal models of absence epilepsy[3]. The seizures occurring in these rats show great similarity with absence seizures in humans in terms of EEG and behavioral characteristics and, responses to antiepileptic drugs[4].

To date, studies that used animal models of absence epilepsy have focused on the role of GABAergic neurotransmission in central cardiovascular regulation. Studies in WAG/Rij and GAERS rats have demonstrated that GABAergic inhibition, especially in the amygdala and hypothalamus, plays a crucial role in the regulation of mean arterial blood pressure and heart rate[5-7]. Another study showed cardiac electrophysiological changes with slower heart rate in GAERS rats and these alterations were due to reduction in cardiac HCN channel expression[8]. However, in the literature, there is no study reporting any impairment of vascular reactivity in absence epileptic animals.

T-type Ca^{++} channels, which are voltage-dependent channels that are activated by low voltage, have been proposed to play an important role in the pathophysiology of absence epilepsy[9]. Ethosuximide (ETX), a non-specific T-type Ca^{++} channel blocker, is still optimal initial treatment for absence epilepsy[10]. It has been reported that ETX is capable of blocking SWDs in WAG/Rij rats[11]. However, to date, the effects of ETX on cardiovascular functions have not been investigated in absence epileptic animals. Thus, we investigated the effects of chronic ETX treatment on blood pressure, heart rate and vascular reactivity in WAG/Rij rats.

MATERIALS AND METHODS

Animals

Eight-weeks old (weighing 100-150 g), male Wistar albino and WAG/Rij rats (Experimental Medical Research and Application Center, Kocaeli University, Turkey) were kept in laboratory conditions with 22±2°C temperature, 45% humidity and 12-h dark/12-h light cycle. Standard chow and tap water were provided ad libitum. All experiments in the current study were conducted according to the Guide for the Care and Use of Laboratory Animals and approved by Kocaeli University Animal Experiments Local Ethics Committee, Turkey (25 March 2014, Number: KOU HADYEK 3/7-2014).

Experimental design

Rats were divided into four groups (n=20): Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups. Control groups did not receive any treatment. ETX groups were treated with ETX (oral, 300 mg/kg/day) for 3 months. The dosage of ETX were selected based on prior works[12,13] which reported that this dosage was effective in blocking SWDs and was also well tolerated without any side effects. ETX was administered by adding it to the drinking water. Prior to experiments, we determined that Wistar rats drink water approximately 100 ml/kg/day and WAG/Rij rats drink approximately 80 ml/kg/day in our pilot measurements. We measured the body weight of the animals regularly. Thus, the dosage of ETX was arranged considering the mean weight and daily water intake volume of animals. At the end of 3-months treatment period, EEG recordings were obtained and then mean arterial blood pressure, heart rate measurements were performed in rats. After that, rats were euthanized and thoracic aortas were isolated for isolated organ bath and immunohistochemical studies.

EEG measurement of SWD

After 3-month-treatment period, rats were anesthetized with ketamine/xylazine (90/10 mg/kg, intraperitoneal). Each rat of the experimental groups was placed in a stereotaxic instrument. The scalp was longitudinally incised and tripolar recording electrodes were implanted into occipital cortex (L -2.0 mm and AP -6.0 mm from bregma), frontal cortex (L -3.5 mm and AP +2.0 mm from bregma)

and cerebellum (for the reference electrode). Electrodes were fixed to the skull with two screws and then animals were allowed to recover for 1 week. After recovery, EEG recordings were obtained from animals for 3 hours (between 9 am and 12 pm) over three consecutive days. The first day was considered as habituation period. The mean values of the recordings of second and third day were assessed. The total, mean duration and number of SWDs were calculated using EEG recordings. The software package LabChart7 was used to analyze the electrophysiological data.

Blood pressure and heart rate measurements

After EEG recordings, a polyethylene catheter (P-10 fused to P-50, Plastic One) filled with heparin/saline (0.5 U/l) solution was placed into the femoral artery of the rats under ether anesthesia. After the catheter was fixed within the vessel, it was removed subcutaneously from the back of the neck. Animals were observed in a plexiglass cage (25x25x30 cm) for 1 hour. Blood pressure was measured using a pressure transducer (FT03, Biopac Systems, Inc.) connected to a data acquisition system (MP100, Biopac Systems, Inc.). Mean arterial blood pressure was evaluated as 1/3 pulse pressure + diastolic pressure. Also heart rate was determined.

Vascular reactivity

The animals were euthanized and thoracic aortic tissues were rapidly isolated and placed in Krebs solution of the following composition (mM): NaH_2PO4, 1.33; $MgCl_2$, 1.05; KCl, 4.71; NaCl, 118; $NaHCO_3$, 25; glucose, 5.6 and $CaCl_2$, 2.7. They were isolated from surrounding tissue, cut into rings and mounted under 1 g tension in organ baths filled with oxygenated (95% O_2 and 5% CO_2) Krebs solution at 37 °C (pH 7.4). All rings were rinsed with Krebs solution at 15-min intervals and equilibrated for 1 hr before the experiments. The isometric tension was measured with a force transducer (FT03, Commat Ltd.) and recorded on a computer by a data acquisition system (TDA 94, Commat Ltd.).

Agonist-induced contractions

In order to test the viability of the tissues, initially, thoracic aortic rings were exposed to KCl (80 mM). Afterwards, the tissues were then washed with Krebs solution and allowed 15 minutes for recovery to restore tension to the precontracted level. After 15 minutes, the aortic rings were contracted with phenylephrine (10^{-8}-10^{-4} M) to obtain a cumulative concentration-response curve. The applied concentrations of phenylephrine were chosen to achieve 80–85% of the maximal response to phenylephrine. The tissues were then contracted by submaximal phenylephrine concentration (10^{-6}-$3x10^{-6}$ M).

Agonist-induced relaxations

Firstly, contractile responses induced by phenylephrine (10^{-6}, $3x10^{-6}$ M) were obtained in aortic rings. When the contraction induced by phenylephrine reached a plateau, in a series of experiments, the concentration-response curves for carbachol (10^{-8}–10^{-5} M), sodium nitroprusside (SNP) (10^{-9}–10^{-4} M) and papaverine (10^{-4} M) were obtained. The tissues were rinsed and allowed to recover for 30 minutes to return the tension to the basal levels between concentration–response curves. The relaxation responses induced by agonists were expressed as percentage of phenylephrine-induced contractile responses.

Immunohistochemical analyses

Thoracic aortic tissues were isolated, fixed with neutral-buffered formalin (10%) and embedded in paraffin. The tissues were separated into 3-μm-thick sections, which were deparaffinized with xylene and hydrated with ethanol. After that, the sections were placed in citrate buffer (1 mM, pH 6.0). Antigen retrieval was performed in a microwave oven followed by an incubation with H_2O_2 (3%) in methanol. Afterwards, sections were incubated with a primary eNOS antibody (sc654, Santa Cruz), followed by an incubation with a secondary biotinylated antibody, streptavidin–peroxidase and diaminobenzidine solution. After the sections were exposed to hematoxylin, they were mounted on glass slides. The slides were evaluated under a light microscope (Leica DM 1000) and photographs were obtained using Leica DMC 2900. Two independent observers, who were blinded to the current study, graded the staining intensity on a semiquantitative scale as follows: no expression: (−), very weak expression: (1+), moderate expression: (2+), strong expression: (3+), very strong expression: (4+).

Solutions and drugs

The all chemicals used in functional studies were

obtained from Sigma-Aldrich and dissolved in distilled water. ETX was also purchased from Sigma-Aldrich and dissolved in drinking water. All drugs and chemicals were prepared rapidly prior to use.

Statistical analysis

Data was presented as mean ± standard error of the mean (SEM). The GraphPad Prism software was used for statistical analysis. The total duration, mean duration also number of SWDs were assessed with the Student's *t*-test for the comparison between two groups. The mean arterial blood pressure, heart rate and functional data were evaluated by one-way ANOVA with a post hoc Bonferroni's test to compare multiple groups. The scores of immunoreactivity were analyzed using nonparametric Kruskal–Wallis test plus Dunn's multiple comparison test, which compare three or more unmatched groups. $p<0.05$ was assumed to be statistically significant. pD_2 and E_{max} values were calculated to evaluate the effects of KCl, carbachol, SNP and papaverine on experimental groups.

RESULTS

The total and mean duration, also number of SWDs were lower in the WAG/Rij ETX group in comparison to WAG/Rij control group ($p<0.01$, $p<0.0001$, $p<0.001$ respectively; Table 1). SWD activity was not found in Wistar control group and Wistar ETX group.

Table 1. The duration and number of SWDs for the WAG/Rij control and WAG/Rij ETX groups

	WAG/Rij control	WAG/Rij ETX
Total duration of SWDs	346.40 ± 58.87	61.28 ± 13.60**
Mean duration of SWDs	6.17 ± 0.35	3.12 ± 0.41****
Number of SWDs	54.29 ± 8.58	12.47 ± 2.54***

Values represent the mean ± SEM. n=16-19 for all groups; **p<0.01, ***p<0.001 and ****p<0.0001 vs. WAG/Rij control group.

Table 2. E_{max} values for carbachol, sodium nitroprusside and papaverine and, pD_2 values for carbachol and sodium nitroprusside in the rings of thoracic aorta obtained from all groups of rats.

	Wistar control	Wistar ETX	WAG/Rij control	WAG/Rij ETX
Carbachol				
E_{max}	82.36 ± 2.70	60.88 ± 4.25***	93.75 ± 1.26**	92.13± 0.93*
pD_2	6.43 ± 0.07	6.52 ± 0.07	6.59 ± 0.04	6.83 ± 0.09
SNP				
E_{max}	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00± 0.00
pD_2	7.02 ± 0.13	7.09 ± 0.15	7.06 ± 0.15	7.13 ± 0.08
Papaverine				
E_{max}	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00± 0.00

Values represent the mean ± SEM. E_{max} values are expressed as percentage of phenylephrine-induced contraction. n=8 for all groups. *p<0.05, **p<0.01 and ***p<0.001 vs. Wistar control group

Table 3. Semi-quantitative distribution of eNOS immunoreactivity in the endothelium of the thoracic aortic tissues obtained from Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups

Animal	Wistar control	Wistar ETX	WAG/Rij control	WAG/Rij ETX
1	+2	+1	+3	+4
2	+3	+2	+4	+4
3	+2	+2	+3	+3
4	+2	+1	+2	+4
5	+2	+2	+3	+2
6	+2	+1	+4	+3
7	+3	+1	+3	+3
8	+3	+1	+4	+4

The staining intensity was classified as no expression (−), very weak (1+), moderate (2+), strong (3+) to very strong (4+) expression

We observed a significant increase in mean arterial blood pressure in WAG/Rij control animals in comparison to Wistar controls (p<0.001; Fig.1). The mean arterial blood pressure of ETX-treated WAG/Rij rats was not statistically different from those of the WAG/Rij control rats and Wistar control rats (Fig.1). It also did not influence the mean arterial blood pressure in Wistar rats (Fig.1).

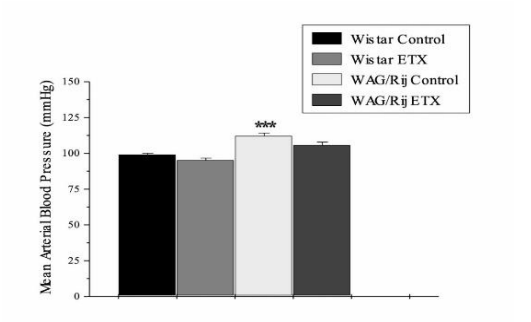

Figure 1. Mean arterial blood pressure (mmHg) in Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups.

***p<0.001 vs. Wistar control group. Values are represented as the mean ± SEM. n=8-10.

There was no significant difference between Wistar control and WAG/Rij control groups in terms of heart rate (Fig.2). ETX treatment significantly decreased the heart rate in Wistar rats (p<0.01; Fig.2). However, the heart rate did not change in ETX-treated WAG/Rij animals in comparison to the WAG/Rij controls (Fig.2).

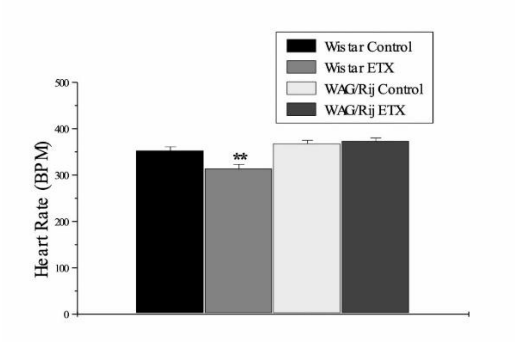

Figure 2. Heart rate (beats per minute (BPM)) in Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups.

**p<0.01 vs. Wistar control group. Values are represented as the mean ± SEM. n=8-10.

The contractile response induced by KCl was markedly found to be diminished in WAG/Rij control rats in comparison to Wistar control rats (p<0.05; Fig. 3). ETX treatment did not influence KCl-induced contractile responses in WAG/Rij rats. (Fig. 3). However, these contractile responses markedly increased in ETX-treated Wistar rats in comparison to Wistar controls (p<0.001; Fig. 3).

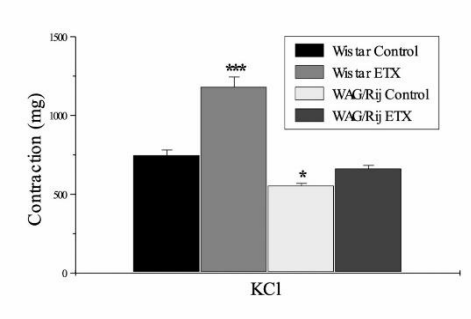

Figure 3. KCl-induced contractile response of thoracic aortic rings obtained from Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups.

*p<0.05 and ***p<0.001 vs. Wistar control group. Values are represented as the mean ± SEM. n=8.

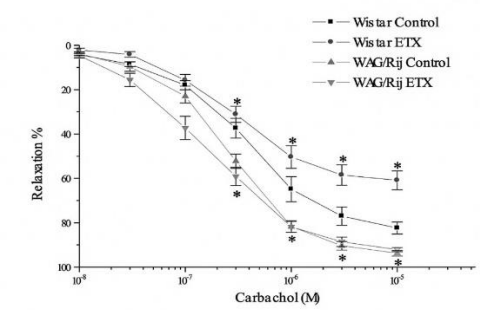

Figure 4. Carbachol-induced relaxation response of thoracic aortic rings obtained from Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups.

*p<0.05 vs. Wistar control group. Values are expressed as percentage of phenylephrine-induced contraction and represented as the mean ± SEM. n=8 for all groups.

Phenylephrine contractile responses were similar between all groups. These tensions were 838.5 ± 45.96 mg, 839.9 ± 81.94 mg, 653.5 ± 41.32 mg and 638.3 ± 54.35 mg in the Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups, respectively. To assess the endothelial function, carbachol-mediated endothelium-dependent relaxation responses was assessed in thoracic aortas from all groups. These responses were markedly increased in WAG/Rij control rats in comparison to

Wistar controls (p<0.05; Table 2, Fig. 4). ETX treatment did not alter carbachol-mediated relaxation responses in WAG/Rij rats (p<0.05; Table 2, Fig. 4).

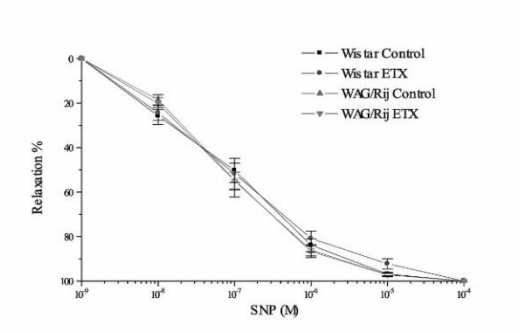

Figure 5. SNP-induced relaxation response of thoracic aortic rings obtained from Wistar control, Wistar ETX, WAG/Rij control and WAG/Rij ETX groups.

Values are expressed as percentage of phenylephrine-induced contraction represent the mean ± SEM. n=8 for all groups.

eNOS immunoreactivity was detected in the endothelial cells of thoracic aortic tissues obtained from experimental groups (Fig. 6). Immunopositivity markedly increased in WAG/Rij control animals in comparison to Wistar controls (p<0.05; Table 3, Fig. 6). ETX treatment significantly decreased eNOS expression in Wistar rats (p<0.05; Table 3, Fig. 6), while it did not affect eNOS expression in WAG/Rij rats (p<0.05; Table 3, Fig. 6).

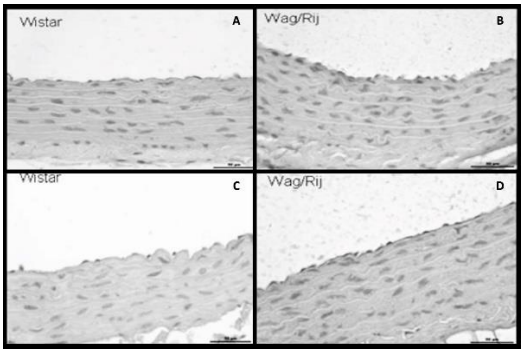

Figure 6. Representative photomicrographs of the endothelium of the thoracic aortic tissues for eNOS immunoreactivity in Wistar control (a), WAG/Rij control (b), Wistar ETX (c) and WAG/Rij ETX (d) groups. n=8 for all groups.

However, ETX treatment resulted in a significant decrease in these responses in Wistar rats (p<0.05; Table 2, Fig. 4). No significant difference was observed between pD_2 values for carbachol in all groups (Table 2). SNP-induced endothelium-independent relaxations in aortic rings did not alter markedly among the experimental groups with no differences in E_{max} and pD_2 values (Table 2, Fig. 5). Additionally, the relaxation responses in response to papaverine did not change among all groups (Table 2).

DISCUSSION

It has been shown that thalamocortical network plays a crucial role in the formation and enlargement of SWDs occurring both in patients with absence seizures and in different animal models of absence epilepsy[14]. T-type Ca^{++} channels, which contribute to the modulation of neuronal firing and formation of neuronal oscillations[15], are highly expressed in the thalamocortical circuit[9]. Studies demonstrated that mice lacking T-type Ca^{++} channels were resistant to SWD occurrence by pharmacological agents[16] and overexpression of T-type Ca^{++} channel lead to the formation of characteristic absence phenotype in mice[17]. In our study, 3-month chronic oral ETX treatment (300 mg/kg/day) administered to WAG/Rij rats suppressed seizures as indicated by a significant decrease in the total and mean duration also number of SWDs. Our findings are partially compatible with the studies which showed that chronic (from postnatal day 21 until 5 months) ETX treatment (300 mg/kg/day) completely blocked the seizures in WAG/Rij rats[12,13]. It has been reported that the first SWDs appearing on cortical EEG recordings in WAG/Rij rats begin to appear at age of 2 months[18]. In our study, we started to give ETX treatment to WAG/Rij rats when they were 2-month-old, which may be the reason for why ETX did not completely block the seizures. Our findings are in agreement with other study which showed that the total duration, mean duration and number of SWDs were markedly suppressed in ETX-treated WAG/Rij rats (80 mg/kg/day, for approximately 3.5 months up to 5 months) compared to that of control rats[11]. Our results confirmed the idea that T-type Ca^{++} current plays a critical role in absence epilepsy.

In our study, arterial blood pressure and heart rate were measured in animals. According to our findings, no significant difference was determined in the mean arterial blood pressure of Wistar rats receiving ETX treatment compared to Wistar control group. Previous studies reported no significant difference

between T-type Ca^{++} channel knock-out mice and controls in terms of mean arterial blood pressure[19,20]. These studies revealed that T-type Ca^{++} channels do not have a direct effect on blood pressure regulation. Our study supported these findings and showed that non-specific pharmacological blockade of T-type Ca^{++} channels with ETX did not influence the mean arterial blood pressure in Wistar rats. However, we found slower heart rate in ETX-treated Wistar rats, indicating that T-type Ca^{++} channels may affect the regulation of heart rate. Similarly, a study demonstrated reduced heart rate in mice lacking $Ca_v3.1$ T-type Ca^{++} channels, suggesting that $Ca_v3.1$ T-type Ca^{++} channels might be involved in heart rate regulation by affecting pacemaker activity and atrioventricular conduction[21]. Also, our findings consistent with another study which reported that mice lacking $Ca_v3.1$ T-type Ca^{++} channels had slower heart rate with no difference in mean arterial blood pressure[19]. This may be attributed to reflex bradycardia which compensate for higher peripheral vascular resistance. Moreover, a significant increase was observed in the mean arterial blood pressure in WAG/Rij control animals in comparison to Wistar controls, but no difference among the two groups was found in heart rate. Our results were parallel to a previous report showing enhanced mean arterial blood pressure with no change in heart rate in GAERS rats compared to Wistar control rats[7]. The increment in the mean arterial blood pressure of WAG/Rij rats might be dependent on an adaptive alteration in response to environmental challenge or dependent on absence seizures observed in this genetic model. However, the reasons for why WAG/Rij rats have higher mean arterial blood pressure levels should be further investigated. Moreover, ETX treatment had no effect on mean arterial blood pressure and heart rate in WAG/Rij rats. The lack of an effect of ETX on mean arterial blood pressure values in WAG/Rij rats or Wistar rats supports our view that T-type Ca^{++} channel blockade has no direct contribution to the regulation of mean arterial blood pressure.

It is well established that in intact endothelium, carbachol or acetylcholine causes an endothelium-dependent vasorelaxation by activating NO/cGMP pathway in vascular smooth muscle cells that are precontracted with noradrenaline, potassium or other vasoconstrictor agents such as phenylephrine[22]. In the present study, endothelium-dependent relaxations in thoracic aortic tissues induced by carbachol in WAG/Rij control animals were significantly higher than Wistar controls. This finding were surprising because we found enhanced blood pressure in WAG/Rij control rats. However, blood pressure is influenced by many factors such as cardiac output, systemic vascular resistance, arterial stiffness and varies depending on situation, emotional state, activity, and relative health/disease states. Moreover, cardiovascular center plays a crucial role on providing neural regulation of blood pressure. On the other hand, thoracic aortas do not directly reflect the peripheral resistance because they are large vessels. Therefore, all of the aforemenationed parameters may affect the blood pressure in WAG/Rij rats. The increased endothelium-dependent relaxations may be due to augmented synthesis and/or release of NO, enhanced vascular smooth muscle responsiveness to NO or increased activation of the mechanisms underlying vascular smooth muscle relaxation in WAG/Rij rats. Moreover, we found increased eNOS expression in thoracic aortic tissues of WAG/Rij control rats compared with Wistar control rats, indicating that increased NO synthesis in thoracic aorta may be the reason for enhanced endothelium-dependent relaxation in WAG/Rij rats. ETX treatment did not affect endothelium-dependent relaxation in WAG/Rij rats but surprisingly, endothelium-dependent relaxation in Wistar rats dramatically reduced by treatment with ETX. This impairment could be associated with diminished NO production in thoracic aortic tissues because we observed markedly reduced eNOS expression in ETX-treated Wistar rats. However, the maximum vasorelaxation induced by SNP (NO donor) and papaverine in endothelium-intact aortic tissues did not change markedly between all groups, indicating that vascular smooth muscle responsiveness to NO was not affected by absence seizures or ETX treatment.

The presence of two subtypes of T-type Ca^{++} channels ($Ca_v3.1$ and $Ca_v3.2$) has been shown in the vascular structure[23,24] and these channels have been suggested to be involved in the excitation-contraction process in rodents and human blood vessels[23,25]. It was demonstrated that T-type $Ca_v3.1$ channels were co-localized with eNOS and promoted NO formation by directly activating eNOS in the endothelium of mouse mesenteric arteries[19]. However, in the same study there were no significant difference between $Ca_v3.1$ channel deficient mice and controls in terms of acetylcholine-induced relaxation responses. In this respect, the contradictory results may be due to many reasons. Firstly, in our study,

blockade of T-type Ca^{++} channel activity was provided by ETX, which was chronically administered to the animals and it has not yet been shown that ETX inhibited which T-type Ca^{++} channel. In addition, we performed our experiments on thoracic aorta and contractions were provided with alpha receptor agonist phenylephrine. However, our results demonstrated that T-type Ca^{++} channel blockade by ETX impairs endothelium-dependent relaxation of thoracic aorta by decreasing NO production or release in Wistar rats, supporting the view that T-type Ca^{++} channels contributes to endothelium-dependent NO-mediated vasodilatation. Accordingly, supporting our findings, another report indicated that T-type Ca^{++} channels play a crucial role in endothelium-dependent relaxation in mice[26]. The increase in endothelium-dependent NO-mediated vasodilation in WAG/Rij rats may be attributed to the hyperactivation of T-type Ca^{++} channels not only in the brain but also in the thoracic aortic tissues. On the other hand, endothelium-dependent NO-mediated vasodilatation in ETX-treated WAG/Rij animals did not differ from WAG/Rij controls. An unknown mechanism different from the activation of T-type Ca^{++} channels may be considered to be responsible for endothelium-dependent NO-mediated vasodilatation in WAG/Rij rats.

In our study, Wistar animals treated with ETX were found to have increased KCl-induced contraction responses in thoracic aortic tissues compared to controls. This enhancement may be dependent on blockade of T-type Ca^{++} channels in Wistar rats. Also, we observed diminished KCl-induced contraction response in WAG/Rij control animals compared to Wistar controls, which might be attributed to the hyperactivation of T-type Ca^{++} channels in WAG/Rij rats. But further investigations are needed to test this hypothesis.

Our study has some limitations that need to be considered. First, we could not provide a direct evidence of T-type Ca^{++} channel activation in the vessels of animals. To determine the role of T-type Ca^{++} channels on the vascular reactivity, patch-clamp recordings of channels could be performed to study ion channel activity. Moreover, measuring the T-type Ca^{++} channel expressions in cardiovascular system may at least in part elucidate the relationship between the channels and observed cardiovascular changes in rats. Additionally, further work is clearly needed to better understand the underlying mechanisms of the increased blood pressure in WAG/Rij rats. Ultimately, studies with additional absence epileptic animal models, and with other drugs that inhibit T-type Ca^{++} channels will be crucial to determine how widely applicable our results are.

In conclusion, we found increased blood pressure and endothelium-dependent vasorelaxations in genetic absence epileptic WAG/Rij rats, while T-type Ca^{++} channel blockade with ETX did not affect these alterations in these rats. However, ETX treatment caused changes in heart rate and endothelium-dependent vasorelaxations in Wistar rats, indicating that T-type Ca^{++} channels might play a role in these changes but further investigations are needed.

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