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The effects of L type calcium channels on the electroencephalogram recordings in WAG/RIJ rat model of absence epilepsy

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Abstract. – BACKGROUND: Epilepsy is one of the most important central nervous system disorder and 1% of the total world population suffers from this disorder which require a chronic drug treatment. Most of the researchers suggested that excessive calcium entry into neurons is the main triggering event in the initiation of epileptic discharges but the role of L type calcium channels has not been clarified in absence epilepsy.

AIM: In this study, it is aimed to investigate the antiepileptic effects of nifedipine, an L type calcium channel blocker and BAY K8644, an L type calcium channel opener in a genetic model of absence epilepsy in WAG/Rij rats.

MATERIALS AND METHODS: Thirty two WAG/Rij rats were allocated into four groups; sham (only saline injected), only nifedipine (an L type calcium channel blocker) injected group (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl), only BAY K8644 (1,4 Dihydro-2,6-dimethyl-5-nitro-4-trifluoromethyl- phenyl-3-pyridine carboxylic acid methyl ester) (L-type C^{a2+}-channel activator) injected group (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl) and combination of their most effective doses BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) injected group. All agents were given by intracerebroventricular injection. The beta, alpha, theta and delta wave ratios of electroencephalogram recordings and the frequency and duration of SWDs (spike and wave discharges) were analyzed and compared between four groups.

RESULTS: Nifedipine increased the number and duration of spike wave discharges whereas BAY K8644 decreased both of them. When BAY K8644 was given after nifedipine, there was no significant difference with control group.

CONCLUSIONS: L type calcium channels play an activator role on spike wave discharges and have positive effects on the duration and frequency.

Key Words:
Epilepsy, WAG/Rij rats, L type calcium channels.

Introduction

Epilepsy is one of the most important central nervous system disorder and 1% of the total world population suffers from this disorder which require a chronic drug treatment. Epileptic seizures are caused by the synchronized and abnormal discharges of the particular neuronal groups[1]. Half of the patients take only symptomatic treatment which suffers from uncontrolled seizures because of the unclear underlying mechanisms of epilepsy[2,3].

Typical absence epilepsy is characterized with spike wave discharges (SWDs) that arise from synchronous firing of thalamocortical networks in electroencephalogram (EEG) and generally seen between 4-10 ages[2]. Absence seizures are typically short in duration and manifest themselves as sudden behavioral arrest and impaired consciousness followed by sudden termination and return to normal behavior[4]. It is important to clarify the pathophysiology of absence epilepsy because clinical studies in absence epileptic patients have shown that these patients have cognitive impairments including general cognitive decline[5], visiospatial dysfunction[5,6], linguistic problems[7], non-verbal and short-term verbal memory impairment[6,7] and attentional, emotional and behavioral alterations[8-11].

WAG/Rij rats were originally developed as an animal model of human absence epilepsy and share many EEG and behavioral characteristics resembling absence epilepsy in humans, including the similarity of action of various antiepileptic drugs[12].

Calcium channels are generally classed as either high voltage-activated or low voltage activated, depending on whether they open at more posi-

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tive or more negative membrane potentials High voltage activated channels can be further classified according to their pharmacological sensitivities and genetic 1 subunit protein composition into L-type, P/Q-type, N-type and R-type[13]. It is suggested that excessive calcium entry into neurons is the main triggering event in the initiation of epileptic discharges[14]. Although the role of other types of high voltage activated channels have been shown clearly in epileptic seizures[15,16], the role of L type calcium channels in absence epilepsy has not been clarified. Thus, in this study, we aimed to investigate the role of L type calcium channels in epileptogenesis by using nifedipine, an L type calcium channel blocker and BAY K8644, an L type calcium channel opener and their combination in WAG/Rij rats, an absence epilepsy model, by recording electroencephalogram.

Materials and Methods

Animals and Reagents

Thirty two WAG/Rij rats weighing 210 ± 30 g at the age of 12-16 weeks were randomly assigned to one of four groups (n=8 each group); sham (only saline injected), only nifedipine (an L type calcium channel blocker) injected group (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl), only BAY K8644 (1,4 Dihydro-2,6-dimethyl-5-nitro-4-trifluoromethylphenyl-3-pyridine carboxylic acid methyl ester) (a neuronal N type calcium channel blocker) injected group (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl) and combination of their most effective doses BAY K8644 (60 µg/2µl) after nifedipine (60 µg/2µl) injected group. All agents were given by intracerebroventricular injection.

Surgery

The rats in the experimental group were anesthetized with a combination of xylazine (3 mg/kg) and ketamine (90 mg/kg) given subcutaneously. Following anesthesia, a small area on the top of the rat's head was shaved and the area was cleaned with betadine. The rat was placed into a small animal stereotaxic apparatus. After a small midline incision on top of the head, the periosteum was removed, the stainless steel screw electrodes were implanted on the dura mater over the cortex, two in the frontal region (coordinates with skull surface flat and bregma zero-zero: AP +1.9; L ±1.5; 1.5 mm below the dura mater) and third one on the occipital region. Electrodes were attached to the skull with dental acrylic. Following surgery, animals were housed in separate cages at a temperature-controlled facility of 23±2°C, a 12-h light/dark cycle (7 a.m. to 7 p.m.) and free access to water and food for 3 days with 4 mg/kg subcutaneous carprofen for analgesia. All animals were euthanized by anesthesia at the end of experimental procedure. All procedures were approved by the Cumhuriyet University Animal Ethics Committee.

Experimental Protocol

Before the experimental protocol, the rats were placed singly in a plexiglas cage, connected to EEG leads, and were habituated to the experimental conditions for 1 h. All baseline and test recordings were performed from 10:00 to 15:00 h to minimize circadian variations. The rats were attached to a multichannel amplifier (EMKA Technologies, Paris, France) via the flexible recording cable. The EEG was continuously recorded in freely moving rats for totally 5 hours, one hour before any injection, one hour after solvent injection and 3 hours after the agent injections in experimental groups and saline injection in sham group. EEGs were displayed on a computer by the IOX 2.4.2.6 Software System (EMKA Technologies, Paris, France). The EEGs were amplified and filtered between 1 and 100 Hz, digitized at 200 Hz and stored for off-line analyses. SWDs were detected manually in WAG/Rij rats. Numbers and durations of SWDs over 20-min time periods were calculated.

The beta, alpha, theta and delta wave ratios of EEG recordings and the frequency and duration of SWDs were analyzed and compared between four groups.

Statistical Analysis

A repeated measures one-way analysis of variance (ANOVA) followed by post hoc Tukey test was used for statistical analysis. The level of statistical significance was considered to be $p < 0.05$. Independent *t*-test was used to assess comparisons between groups. Each EEG recording was divided into 20-min epochs, and the duration and number of SWDs were analyzed separately for each epoch; the resulting data were expressed as means ±SEM for each time point.

Results

There was not any significant difference between the basal beta, alpha, and delta wave ratios

of EEG recordings of three experimental groups (Figure 1). Beta and delta recording ratios in 60 µg/2 µl nifedipine injected group was significantly different from basal and other doses injected animals' recordings ($p < 0.05$) (Figure 2). Beta and alpha recording ratios in 60 µg/2 µl BAY K8644 injected group was significantly different from basal and other doses injected animals' recordings ($p < 0.05$). Moreover, theta and delta recording ratios in 60 µg/2 µl and 80 µg/2 µl BAY K8644 injected groups were significantly different from basal and 40 µg/2 µl dose injected groups ($p < 0.05$) (Figure 3). In BAY K 8644 after nifedipine group, to investigate combination effects of the most effective doses of both agents (60 µg/2 µl and 60 µg/2 µl, respectively) were used. Nifedipine changed all wave ratios significantly ($p < 0.05$), but this significance was abolished in beta, theta and delta wave ratios after BAY K 8644 injection (Figure 4).

In sham group, there was no significant difference of frequency and duration of SWDs after saline injection. Nifedipine and BAY K8644 significantly decreased the frequency and duration of SWDs ($p < 0.05$). There was not any significant difference between the frequency and duration of SWDs of basal and BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) group recordings (Figure 5).

Discussion

The different types of calcium channel 1 subunits are differentially distributed within neurons and P/Q-type, N-type, and R-type channels are expressed highly at presynaptic nerve terminals where their activities evoke neurotransmitter release[17]. T-type calcium channels are expressed at cell bodies and dendrites[18] and they contribute to the regulation of neuronal excitability[19]. Taken together, P/Q-type and T-type calcium channels show distinct functional properties, subunit composition, and subcellular distributions, and they serve distinct physiological roles and they both are major contributors to the development of absence seizures and idiopathic generalized epilepsies[16].

The increase of intracellular and the decrease of extracellular levels of calcium during an epileptic seizure have been shown by Pelletier et

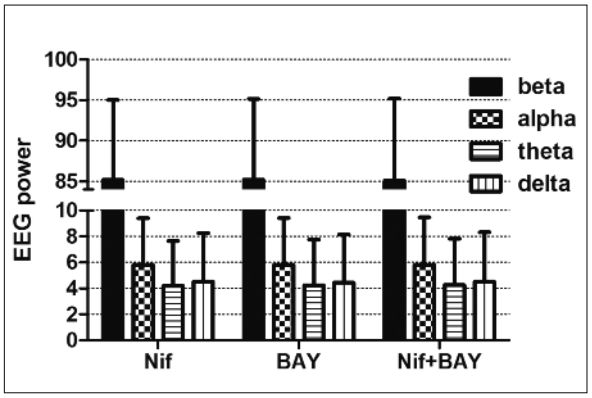

Figure 1. Comparison of basal beta, alpha, theta and delta wave ratios of EEG recordings (Nif: Nifedipine, BAY: BAY K8644).

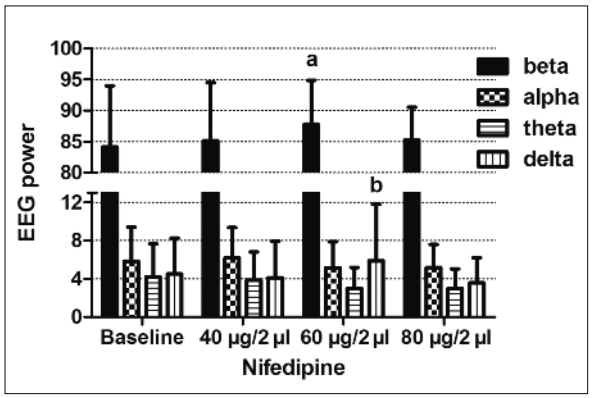

Figure 2. Comparison of beta, alpha, theta and delta wave ratios of EEG recordings after different doses of nifedipine (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl) intracerebroventricular injection. [a,b]Statistically different from basal, 40 µg/2 µl and 80 µg/2 µl nifedipine injected groups ($p < 0.05$).

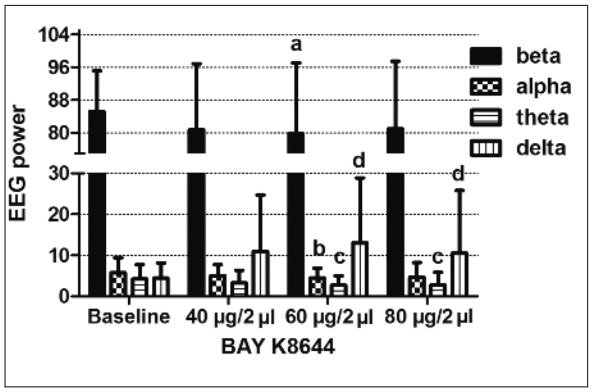

Figure 3. Comparison of beta, alpha, theta and delta wave ratios of EEG recordings after different doses of BAY K8644 (40 µg/2 µl; 60 µg/2 µl; 80 µg/2 µl) intracerebroventricular injection. [a,b]Statistically different from basal, 40 µg/2 µl and 80 µg/2 µl BAY K8644 injected groups ($p < 0.05$). [c,d]Statistically different from basal and 40 µg/2 µl BAY K8644 injected groups ($p < 0.05$).

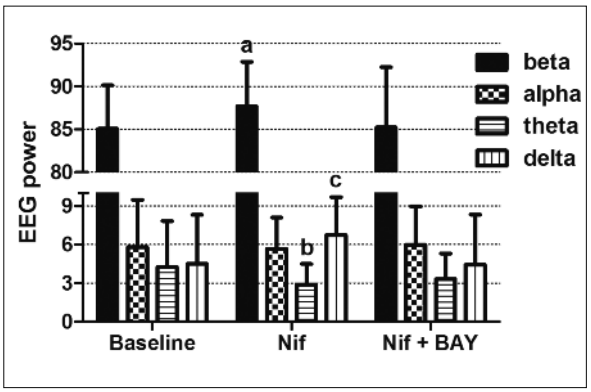

Figure 4. Comparison of beta, alpha, theta and delta wave ratios of EEG recordings of BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) intracerebroventricular injections (Nif: Nifedipine, BAY: BAY K8644). [a,b,c]Statistically different from basal and BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) injected groups ($p < 0.05$).

al[20]. The inhibition of calcium influx by anti-convulsive drugs such as phenytoin and carbamazepine has been reported[21]. An antiepileptic agent, verapamil, is a specific blocker of L-type calcium channels and have marked anticonvulsive effects. All these results show that calcium channel blockers and antagonists have antiepileptic effects by inhibiting intracellular calcium influx and calcium channel activation[21,22].

Although, calcium channel blockers are generally accepted as anticonvulsive[21], T and L type calcium channel blockers have shown contrary effects in non-convulsive epilepsy[23]. Besides, the blockers and openers of these channels have peripheral effects of skeletal and cardiac system such as blood pressure. According to this peripheral effects these agents may affect the seizure activity like spike wave discharges indirectly[24].

It has been shown that nimodipine, an L type calcium channel blocker, increase the frequency in acute and chronic experiments[25]. BAY K8644 is generally accepted as an pro-convulsive agent in most of the experimental epilepsy models. It did not triggered the audio genic seizures in rats, is not effective in convulsion threshold test in rats and convulsions may be seen after high doses[26]. Fatal convulsions have been seen in old WAG/Rij rats. Generally, the pro-convulsive effects of BAY K8644 are less than its effects on SWDs[24]. As a result, the contrary effects of BAY K8644 in convulsive and non-convulsive epilepsy like nimodipine have been shown and these effects cannot be explained by its peripheral effects[27,28].

Although there are many studies investigating the role of calcium channels in epileptic seizures[15,16], the role of L type calcium channels is elusive especially in absence epilepsy which causes clinical problems such as cognitive impairments including general cognitive decline, visiospatial dysfunction, linguistic problems, non-verbal and short-term verbal memory impairment, and emotional and behavioral alterations[5-11].

In this study the role of an L type calcium blocker (nifedipine) and an opener (BAY K8644)

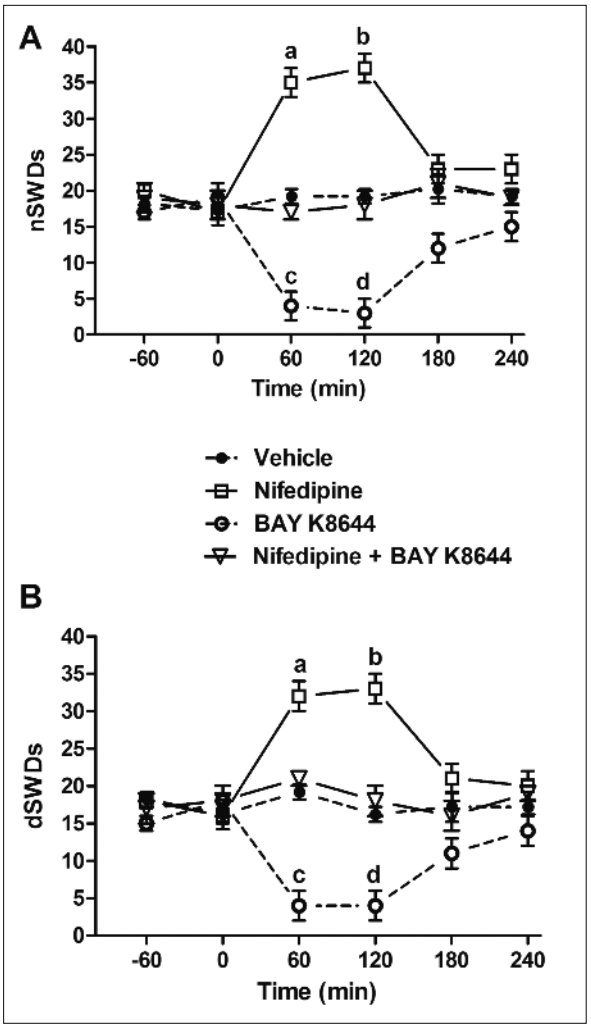

Figure 5. Comparison of frequency nSWDs (A) and duration dSWDs (B) of SWDs of EEG recordings of vehicle, only BAY K8644 (60 µg/2 µl), only nifedipine and BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) intracerebroventricular injections. [a,b]Statistically different from vehicle, only BAY K8644 (60 µg/2 µl) and BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) injected groups ($p < 0.05$). [c,d]Statistically different from vehicle, only nifedipine (60 µg/2 µl) and BAY K8644 (60 µg/2 µl) after nifedipine (60 µg/2 µl) injected groups ($p < 0.05$).

on beta, alpha, theta and delta wave ratios and SWDs have been investigated. Nifedipine significantly changed the beta and delta wave ratios whereas BAY K8644 significantly changed beta and alpha wave ratios. BAY K 8644 reversed the effects of nifedipine on beta, theta and delta wave ratios. Nifedipine increased both of the duration and frequency of SWDs while BAY K8644 decreased. On the other hand, the combination of both agents did not affect the duration and frequency of SWDs.

Conclusions

L type calcium channels play an activator role on SWDs and have positive effects on the duration and frequency. These positive effects may be through their central effects more than peripheral effects. Further experimental and clinical studies with L type calcium channels besides other types of calcium channels may contribute to clarify the pathophysiology of absence epilepsy and develop new treatment strategies.

Declaration of funding Interest

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