Evaluation of effects of T and N type calcium channel blockers on the electroencephalogram recordings in Wistar Albino Glaxo/Rij rats, an absence epilepsy model

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ABSTRACT

Objectives: It is suggested that excessive calcium entry into neurons is the main triggering event in the initiation of epileptic discharges. We aimed to investigate the role of T and N type calcium channels in absence epilepsy experimental model.

Materials and Methods: Wistar Albino Glaxo/Rij (WAG/Rij) rats (12–16 weeks old) were randomly allocated into four groups; sham, mibefradil (T type calcium channel blocker), w-Conotoxin MVIIA (N type calcium channel blocker), and mibefradil + w-Conotoxin MVIIA. Beta, alpha, theta, and delta wave ratios of EEG recordings and frequency and duration of spike wave discharges (SWDs) were analyzed and compared between groups.

Results: Beta and delta recording ratios in 1 μM/5 μl mibefradil group was significantly different from basal and other dose-injected groups. Beta, alpha, and theta recordings in 0.2 μM/5 μl w-Conotoxin MVIIA group was significantly different from basal and other dose-injected groups. In w-Conotoxin MVIIA after mibefradil group, beta, alpha, and theta recording ratios were significantly different from basal and mibefradil group. Mibefradil and w-Conotoxin MVIIA significantly decreased the frequency and duration of SWDs. The decrease of frequency and duration of SWDs in mibefradil group was significantly different from w-Conotoxin MVIIA group. The frequency and duration of SWDs significantly decreased in w-Conotoxin MVIIA after mibefradil group compared with basal, mibefradil, and w-Conotoxin MVIIA groups.

Conclusions: We concluded that both T and L type calcium channels play activator roles in SWDs and have positive effects on frequency and duration of these discharges. These results are related with their central effects more than peripheral effects.

KEY WORDS: Epilepsy, mibefradil, T and N type calcium channels, Wistar Albino Glaxo/Rij rats, w-Conotoxin MVIIA

Introduction

Epilepsy is one of the most important central nervous system disorder that affects the 1% of the world population without demographic difference and requires a chronic drug treatment.[1,2] Comorbid situations during seizure control, drug interactions, and adverse effects make it difficult to find an efficient and cheap treatment. The underlying mechanisms of epilepsy are not clear yet, thus, half of the patients that suffers from uncontrolled seizures take only symptomatic treatment.[3,4] It is suggested that excessive calcium entry into neurons is the main triggering event in the initiation of epileptic discharges.[5,6] Wistar Albino Glaxo/Rij (WAG/Rij) rats, originally developed as an animal model of human absence epilepsy, share many electroencephalography (EEG) and behavioral characteristics resembling absence epilepsy in humans, including the similarity of action of various anti-epileptic drugs.[7]

Many researches about the molecular underlying mechanisms of the epilepsy have been focused on the calcium messenger system and excessive calcium entry into the cell
have been proposed to trigger the epileptic activity.[8] It has been shown that extracellular calcium decreases while intracellular calcium increases during the epileptic seizures.[9] Calcium channel blockers prevent the entry of calcium into the cell and they are used to restrict the ischemia.[10] The modulator relation between calcium blockers and the epilepsy has been shown but the exact role of them in the pathophysiology of epileptic seizures has not been clarified yet.[11] In this study, we aimed to investigate the role of T and N type calcium channels in absence epilepsy by using mibefradil, a T type calcium channel blocker; w-Conotoxin MVIIA, a neuronal N type calcium channel blocker and their combination in WAG/Rij rats, an absence epilepsy model by recording electroencephalogram.

Materials and Methods

Animals

Thirty two 12–16-weeks-old WAG/Rij rats weighing 220 ± 30 g were randomly assigned to one of four groups (n = 8 each group); sham (only saline injected), only mibefradil (a T type calcium channel blocker) injected group (1 μM/5 μl, 2 μM/5 μl, 4 μM/5 μl), only w-Conotoxin MVIIA (a N type calcium channel blocker) injected group (0.1 μM/2 μl, 0.2 μg/5 μl, 0.4 μM/2 μl). In the fourth group, the most effective doses of the both agents were chosen and w-Conotoxin MVIIA (0.2 μM/2 μl after mibefradil (1 μM/5 μl) were injected. All agents were given by intracerebroventricular (icv) injection.

The rats in the experimental groups were anesthetized with a combination of xylazine (3 mg/kg) and ketamin (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-hour 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 and were conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals.

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 hour. All baseline and test recordings were performed from 10:00 to 15:00 hour 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, 1 hour before any injection, 1 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. Spike wave discharges (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 ± scanning electron microscope (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 1 μM/5 μl mibefradil-injected group was significantly different from basal and other dose-injected animal recordings (P < 0.05) [Figure 2]. Beta, alpha and theta recording ratios in 0.2 μM/5 μl w-Conotoxin MVIIA-injected group was significantly different from basal and other dose-injected animals recordings (P < 0.05) [Figure 3]. In w-Conotoxin MVIIA after mibefradil group, the most effective doses of both agents (0.2 μM/3 μl and 1 μM/5 μl, respectively) were used to investigate the combination effects of both T and N type calcium channel blockers. Beta, alpha, and theta recording ratios in this group were significantly different from basal and only 1 μM/5 μl mibefradil-injected group (P < 0.05) [Figure 4].

In sham group, there was no significant change of frequency and duration of SWDs after saline injection. Mibefradil significantly decreased the frequency and duration of SWDs (P < 0.05), this decrease was maximum after 120 min and returned to the basal levels after 180 min. w-Conotoxin MVIIA significantly decreased the frequency and duration

Figure 1: The basal beta, alpha, theta, and delta wave ratios of electroencephalography (EEG) recordings (mibef: Mibefradil, con: w-Conotoxin MVIIA, mibef + con: Mibefradil + w-Conotoxin MVIIA)
of SWDs ($P < 0.05$), this decrease was maximum after 60 min and returned to the basal levels after 180 min. The decrease of frequency and duration of SWDs in only mibefradil (1 μM/5 μl) injected group was significantly different from only w-Conotoxin MVIIA (0.2 μM/5 μl) injected group ($P < 0.05$).

The frequency and duration of SWDs significantly decreased in w-Conotoxin MVIIA (0.2 μM/5 μl) after mibefradil (1 μM/5 μl) group ($P < 0.05$), this decrease was maximum after 60 min and returned to the basal levels after 180 min. This decrease was significantly different from basal, only mibefradil (1 μM/5 μl) and only w-Conotoxin MVIIA (0.2 μM/5 μl) ($P < 0.05$) [Figure 5].

**Discussion**

The effect mechanisms of calcium channels in epileptogenesis are related with their electrophysiological, pharmacological, and bio-physiological effects and all calcium channels (L, T, P/Q, and N) are present in the brain. SWDs are especially originated from thalamus and T type channels are more than L types in this area.[12] SWDs occur after the rhythmic interactions between thalamic and cortical areas and T type calcium channels have active role in these areas. Although there are important queries on the effects of ethosuximide, it is important in the depressing the T type channels in the thalamic neurons.[13] Leresche et al., showed the ineffectivity of ethosuximide on low and high threshold calcium currents and its decreasing effects on the non-active sodium currents which affect the calcium activated potassium currents. These results may explain the burst firing, increase in the tonic firing and SWDs.[14]
It has been suggested that BAY K8644 (calcium channel agonist) and nimodipine (dihydropyridine calcium channel blocker) have two possible mechanisms in their antagonism effects of epileptic activity. Firstly, BAY K8644 may antagonize the epileptiformic effects of nimodipine in non-convulsive epilepsy. Secondly, nimodipine may inhibit convulsions by BAY K8644. Intraperitoneal nimodipine reversed the effects of intraventricular ejected BAY K8644[15] which show the central effects of both agents. These results show the possible effects of L type calcium channels with T types in SWDs[15,16] and the induction of L type channels by T types during the inhibition of SWDs. Although, there are studies investigating the role of L type calcium channels in the firing mode of talamocortical neurons,[17] the role of T and N type calcium channels is not clear in the generalized absence epilepsy.

In this study, we aimed to clarify the effects of T and N type calcium channel blockers on the pathophysiology of absence epileptic seizures by recording electroencephalogram. Beta and delta recording ratios in 1 μM/5 μl mibefradil-injected group was significantly different from basal and other dose-injected animals recordings. Beta, alpha, and theta recording ratios in 0.2 μM/3 μl w-Conotoxin MVIIA-injected group was significantly different from basal and other dose-injected animals recordings. In w-Conotoxin MVIIA after mibefradil group, beta, alpha, and theta recording ratios were significantly from basal and only mibefradil-injected group. Mibefradil and w-Conotoxin MVIIA significantly decreased the frequency and duration of SWDs. The decrease of frequency and duration of SWDs in only mibefradil-injected group was significantly different from the only w-Conotoxin MVIIA-injected group. The frequency and duration of SWDs significantly decreased in w-Conotoxin MVIIA after mibefradil group compared with basal, only mibefradil and only w-Conotoxin MVIIA groups.

Spike wave discharges in electroencephalogram are one of the key symptoms of generalized absence epilepsy. Low-voltage-activated or T type calcium channel flows are thought to play significant role in thalamic neuronal rhythmic firing properties. It is known that talamocortical transmission cells are hyperpolarized during wakefulness to fain.[18-20]

The current amplitude of T type calcium channels were increased in a rat absence epilepsy model developed by Marescaux et al.[21] Ethosuximide, a specific anti-absence drug, blocks T type calcium channels with another mechanism.[22] Ethosuximide is used in the treatment of absence epilepsy and it is known that it blocks the T type calcium channels by inhibiting the calcium entry especially in the thalamic area neurons and low threshold calcium increase is depressed and burst firing is inhibited. The effects of ethosuximide on SWDs have been shown in human and animals by in vivo and in vitro studies.[23] Van Luijtelaar et al.[16] used L and T type calcium channel modulators BAY K8644, nimodipine and ethosuximide in WAG-Rij rats, a genetic absence epilepsy model. They also investigated the role of calcium channel subunits by using w-Conotoxin GVIA and w-Conotoxin MVIIA, an N type calcium channel blocker and a P/Q type calcium channel blocker, respectively. Both of the calcium channel subunits are present in the central nervous system. They showed the relations between the SWDs and P/Q channel subunits in these rats.[24]

Although, calcium channel blockers are generally thought to be anti-convulsive,[21] it has been shown that T and L type calcium channel blockers may have opposite effects in non-convulsive epilepsy.[16] Besides, calcium channel blockers and openers affect the central parameters like blood pressure by their peripheral effects on skeletal and cardiac systems. These agents affect the seizure activity like SWDs related with their peripheral effects.[25]

In conclusion, we have showed that both T and L type calcium channels play activator roles in SWDs and have positive effects on the frequency and duration of these discharges. These results are related with their central effects more than peripheral effects.

Acknowledgement

This study was funded in full by Cumhuriyet University Scientific Project Support Unit, grant number [T-384].

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Cite this article as: Durmus N, Gültürk S, Kaya T, Demir T, Parlak M, Altun A. Evaluation of effects of T and N type calcium channel blockers on the electroencephalogram recordings in Wistar Albino Glaxo/Rij rats, an absence epilepsy model. Indian J Pharmacol 2015;47:34-8.

Source of Support: Nil. Conflict of Interest: No.