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ARAŞTIRMA / RESEARCH

Expression levels of KCNQ1 and KCNQ3 genes in experimental epilepsy model

Deneysel epilepsi modelinde KCNQ1 ve KCNQ3 genlerinin ekspresyon düzeyleri

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Abstract

Purpose: Status epilepticus (SE) is a highly common neurological disease in children, with recurrent generalized convulsions for more than 30 minutes, and when not controlled, neuronal damage occurs in the brain. The aim of this study was to evaluate the changes in KCNQ1 and KCNQ3 gene expression levels in the acute period after SE.

Materials and Methods: In rats, an experimental SE model was created with Li-Pc. Study; female Wistar albino [250–350 g, 21 (n=7)] rats were used in 3 groups as SE, control and sham groups. The total RNA obtained from brain tissues was converted to cDNA, and gene expression levels of KCNQ1 and KCNQ3 genes were assessed by quantitative real-time PCR (qRT-PCR) method.

Results: Statistically in SE group; compared to the control and sham groups, a significant difference was observed in the gene expression levels of the KCNQ1 and KCNQ3 ion channels. The KCNQ1 and KCNQ3 gene expression levels of the experimental group was found higher than the other groups.

Conclusion: Determining the changes in mRNA expression levels of genes encoding K^+ ion channels will help to better understand the pathological mechanisms that occur during epilepsy. In the SE experimental model created, it is believed that an increase in mRNA expression of KCNQ1 and KCNQ3 will lead to drug therapy studies planned for the future.

Keywords:. Epilepsy brain, Li-pilocarpine, status epilepticus (SE), KCNQ1, KCNQ3, rat

Öz

Amaç: Status epileptikus (SE) 30 dakikadan daha uzun süre tekrarlayan jeneralize konvulziyonlarla birlikte çocuklarda oldukça yaygın bir şekilde görülen nörolojik bir hastalıktır ve kontrol edilmediğinde, beyinde nöronal hasarlar meydana gelir. Bu çalışmanın amacı SE sonrası akut dönemde KCNQ1 ve KCNQ3 gen ekspresyon seviyelerindeki değişiklikleri değerlendirmektir.

Gereç ve Yöntem: Sıçanlarda Li-Pc ile deneysel SE modeli oluşturuldu. Çalışmada; SE, kontrol ve sham grupları olmak üzere 3 grupta dişi Wistar albino [250–350 gr, 21 adet (n=7)] sıçan kullanıldı. Beyin dokularından elde edilen total RNA, cDNA'ya çevrilerek KCNQ1 ve KCNQ3 genlerinin kantitatif gerçek zamanlı PCR (qRT-PCR) yöntemi ile gen ekspresyon değişimleri değerlendirildi.

Bulgular: İstatistiksel olarak SE grubunda; kontrol ve sham grubuna kıyasla KCNQ1 ve KCNQ3 genlerinin ekspresyon seviyelerinde anlamlı oranda farklılık görüldü. Deney grubunun KCNQ1 ve KCNQ3 gen ekspresyon düzeyleri diğer gruplardan yüksek bulundu.

Sonuç: K^+ iyon kanallarını kodlayan genlerin mRNA ekspresyon seviyelerindeki değişikliklerin belirlenmesi epilepsi sırasında oluşan patolojik mekanizmaların daha iyi anlaşılmasına yardımcı olacaktır. Oluşturulan SE deneysel modelde KCNQ1 ve KCNQ3 mRNA ekpresyonlarında artış saptanması ileride yapılması planlanan tedaviye yönelik ilaç çalışmalarına yol göstereceğine inanılmaktadır.

Anahtar kelimeler: Epilepsi, beyin, Li-pilokarpin, status epileptikus (SE), KCNQ1, KCNQ3,sıçan

INTRODUCTION

Ion channels play key roles in the production of membrane potential and function in various cellular activities such as signal transduction, neurotransmitter release, muscle contraction, hormone secretion, hydro electrolyte balance, growth, motility and apoptosis[1]. These channels can

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consist of a single protein or a group of some subunits, each of which is a protein encoded by a different gene. About 600 ion channel genes have been recognised. The ion channels can be associated with a variety of hereditary diseases associated with hyperexcitability, particularly epilepsies[2,3].

Epilepsy is a neurological disease characterized by spontaneous and recurrent seizures caused by excessive, abnormal and hypersynchronous neuronal activity[4]. Epilepsy patients carry 18-24 times higher risk of Sudden Unexpected Death in Epilepsy (SUDEP) than the normal population[5]. SUDEP is the sudden and unexpected death of cases where witnesses may or may not be healthy without SE (Status Epilepticus) epilepsy patients from other directions. In SUDEP cases, no cause of death can be detected in postmortem examinations. However previously it was shown that SUDEP may be associated with cardiootonomic-respiratory dysfunction and cardiac arrhythmias. In this context, the relevance between cardiac arrhythmias and epileptic seizures was first shown by Hartmann et al[6]. In this study, the relationship between Na^+ channel (SCN5A) and long QT syndrome was revealed. In the following years, it was concluded that there is a connection between K^+ channels and cardiac arrhythmias[7].

K channels are ion channels encoded by more than 70 genes found in every cell of the organism[8]. Potassium channels are included in the formation and transmission of electrochemical signals in cells[9]. The most known K^+ channels encoded gene associated with epilepsy are KCNQ2 and KCNQ3 and mutations in these genes expressed in the brain are known to cause refractory epilepsies in the neonatal period[10].

The KCNQ1 gene has very similar homology with KCNQ2 and 3 and it has been demonstrated that mutations in this gene cause Long OT syndrome (LQT)[11]. The KCNQ1 gene has been expressed in the brain and its association with epilepsy has been recently demonstrated in a limited number of experimental studies[12].

SE is defined as seizure activity countinies at least 30 minutes without interruption and causes neuronal cell death. It is an emergency that must be intervened due to the risk of mortality[13]. SE causes neuronal damage to the brain tissue through mechanisms such as oxidative stress, inflammation and mitochondrial dysfunction[14]. In in vitro and in vivo investigations, the function of K^+ ion channels in epilepsy has not been fully elucidated. This is the first study to show the changes in K^+ ion channel expression in the epileptic animal model. We hypothesized that detecting variations in the expression of K^+ ion channels would contribute to the development of therapeutic drugs. For this purpose, we investigated the changes in KCNQ1 and KCNQ3 gene expression levels in the acute period after SE.

MATERIALS AND METHODS

Animals model

In this experiment 21 adult female rats were used. The experiment process were permitted by ÇOMÜ Animal Experiments Local Ethics Committee (HADYEK-2020-2000038720). This study was approved by the university ethics committee and all procedures performed in the study were carried out in accordance with the 1964 Helsinki Declaration standards. Along the study, all rats were kept at 23°-25ºC temperature in 12 hours light/12 hours dark rhythm (light, 08:00-20:00, dark 20: 0-08:00).

Chemicals

Pilocarpine hydrochloride and lithium chloride were purchased from Sigma Aldrich. Diazepam and atropine were used from commercial form.

Experimental design

A total of 21 female rats were randomly separated into three groups (n = 7)

Group SE: Only SE was performed and the treatment was terminated at the 24th hour following SE.
Group Control: No intervention was performed. Normal care and nutrition was given.
Group Sham: 2 ml SF application was performed ip for 48 hours with an interval of 12 hours from the beginning of the study.

After sacrifice, rat brains samples were taken for genetic analysis in all groups.

In the experimental model, the pilocarpine (Pi) initia dose was 200 mg/kg/dose (ip) to minimize the mortality rate, and the occurrence of stage 4/5 seizure activity was observed according to the Racine scale. An additional 100 mg/kg/dose of Pi was administered to rats that did not show Racine stage 4/5 seizure activity at the initial dose. In order to

decrease mortality, rats with 60 minutes or more Racine stage 4/5 seizure activity were treated with 5 mg/kg/dose diazepam (ip) to end the seizure. To increase pilocarpine (Pi) sensitivity and reduce lethal side effects, 127 mg/kg/dose Li with physiological saline was administered 24 hours before administration by sc. In addition, 1-5 mg/kg/dose of atropine sulfate (sc) was made 30 minutes before SE induction to reduce peripheral cholinomimetic effects due to Pi.

Stages of Racine scale are as follows;

Stage 1: Around the face and mouth movements, steepening feathers
Stage 2: Head-shaking movements
Stage 3: Clone of the front extremities
Stage 4: Clone of front extremities with rearing and seizure
Stage 5: Cloning of the extremities (generalized motor convulsions) with rearing and subsequent falling and seizure[15-18].

mRNA isolation and quantitative real-time RT-PCR analysis

Genes expressions were evaluated by quantitative real-time RT-PCR. Total RNA was isolated from 10-30 mg brain tissue using the QIAamp RNA column (Ambion Pure Link RNA Mini Kit) according to the kit protocol. The quality and amount of mRNA was determined according to the 260/280 absorbance rate using the Nano Drop ND-1000 Spectrophotometer. Reverse transcription (High Capacity cDNA Revere Transcription Kit) was performed using the cDNA synthesis kit. Synthesized cDNA samples were used for quantitative real-time polymerase chain reaction (qRT-PCR).

Gene expression levels were analyzed by qRT-PCR (ABI Stepone) using Applied Biosystems ™ TaqMan® Gene Expression (Thermo Fisher Scientific, USA). β-actin was used for the normalization of genes. Gene expressions were determined using Ct values and fold changes were evaluated by the $2^{-(\Delta\Delta CT)}$ method. Primer ID numbers for KCNQ1, KCNQ3 and β-actin are Rn00583376_m1, Rn00580995_m1, Rn00667869_mL respectively (Thermo Fisher).

Statistical analysis

All results were assessed by SPSS Statisticsfor Windows, Version 20.0 (Armonk, New York, USA: IBM Corp.) Mean and standard error values were used to present the descriptive data. All groups were compared by One WayAnova test, followed post-hoc Tukey's test and $p<0.05$ was statistically significant. Genes expression level were evaluated $2^{-\Delta\Delta Ct}$ method [ΔΔCt=(Ct Target gene- Ct reference gene).

RESULTS

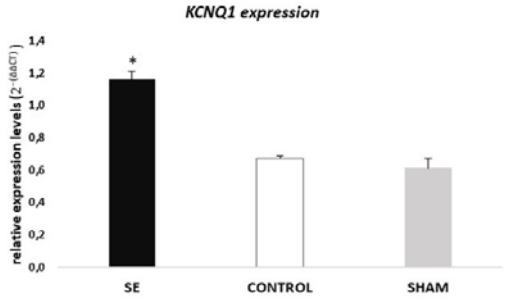

Fig 1. Gen expression levels of KCNQ1 in all groups of rat brain tissue. After normalized them RNA level with β-Actin, it is showed as a $2^{-(\Delta\Delta CT)}$ relative expression.
One-way ANOVA was used to compare gene expressions levels between groups and results were evaluated according to the post-hoc Tukey's test. *:different from Control and Sham groups, $p<0.00$.

Potassium voltage-gated channels subfamily Q member 1 (KCNQ1): Gene expression levels of KCNQ1 in rat brain tissue are presented in Fig 1. SE group was shown to have KCNQ1 gene expression at higher levels than all other groups. This difference was found to be significant with post-hoc analyses ($p<0.00$).

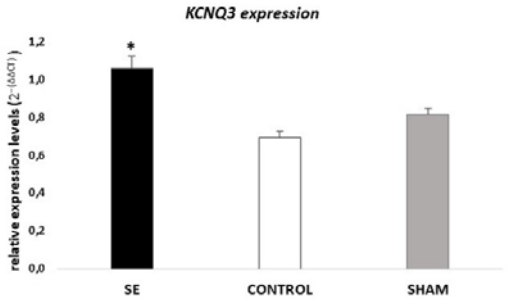

Fig 2. Gen expression levels of KCNQ3 in all groups of rat brain tissue. After normalized them RNA level with β-Actin, it is showed as a $2^{-(\Delta\Delta CT)}$ relative expression.
One-way ANOVA was used to compare gene expressions levels between groups and results were evaluated according to the post-hoc Tukey's test. *:different from Control and Sham groups, $p<0.05$.

Potassium voltage-gated channel subfamily Q member 3 (KCNQ3): KCNQ3 gene expression levels in rat brain tissue are shown in Fig 2. The highest gene expression level was found in the SE group, it was found to be statistically different from the control and sham groups ($p<0.05$). Apart from SE, the KCNQ3 gene expression levels in the groups were similar and no difference was detected.

DISCUSSION

In our study, the mRNA changes of the K^+ ion channels (encoded by KCNQ1 and KCNQ3) in the brain tissue of the SE modeled rats were examined and an increase was observed in the SE group compared to the control and sham groups. This gene expression profile of KCNQ1 and KCNQ3 may reveal their contribution to hyperexcitability. In previous studies, KCNQ1 has been associated with long QT syndrome and deafness and epilepsy[19-21]. KCNQ-mediated potassium flow also called M current, has been reported to be important in regulating neuronal excitability of the central nervous system and a 25% reduction in this current may cause epilepsy[22]. In recent years, gene defects fundamental several forms of epilepsy have been identified and it has been reported that most of the genes encoding these ion channels play an important role in the pathogenesis of idiopathic epilepsy. Various epileptic phenotypes have been connected with mulfunctions of K^+ ion channels. The channels play an essential role in neuronal stimulation, and their significance is correlated to the levels of expression in the subcellular area, cell or domain[23].

There are more than 70 potassium ion channel genes in humans, however some of them have been associated with neurological disorder. Several KCNQ genes mutations are associated with diseases such as cardiac arrhythmia, deafness, and epilepsy show vary physiological roles of KCNQ channels. In addition, several KCNQ isoforms can combine to form heteromeric channels that underlie the M-stream, an important regulator of neuronaleability[24]. Brain-expressing KCNQ5 alone or together with KCNQ3 can form M-type channels and make all these subunits and several heteromers interesting targets for drug development[25].

In different parts of the brain KCNQ2/KCNQ3 channels appear to be the main factor of "M" currents blocked by various neurotransmitters, including acetylcholine (ACh) through muscarinic receptors. K^+ ion channels represent important targets for the new pharmacological control of abnormal electrical discharges and synaptic function in the brain. Therefore, more effort should be studied to find new K^+ ion channel regulators and gene therapies to improve symptoms of the disease[8]. KCNQ1 is expressed in some tissues, including the heart muscle, and many epithelium, but KCNQ1 mutations effect serious outcomes in a little tissue, mainly depending on the degree of current reduction[26].

The four other related channel proteins (KCNQ2, -3, -4 and -5) provided interesting information last years. Mutations in the KCNQ genes form the basis of various diseases and lead to electrical hypersensitivity in cardiac arrhythmia and epilepsy, disorders of transepithelial transport in congenital deafness, and possibly cell degeneration in progressive hearing loss[27]. Although previous studies on the KCNQ family Delmas and Brown et al. describe KCNQ1 channels as non-neuronal channels, gene expression studies clearly demonstrate the expression of KCNQ1 in the central nervous system; Luo et al. showed expression of KCNQ1 in the human brain. Goldman et al. reported the expression of KCNQ1, including glia cells of the white matter tracts in the frontal, temporal, parietal and occipital lobes, as well as in the hippocampus and spinal cord[28-30].

Epilepsy is a brain disorder that frequently generates muscle spasms, convulsions and loss of consciousness by irregular firing of neuronal networks in the brain[8]. Epileptic seizures are stimulated by irregular focal or synchronous electrical discharges within the central nervous system (CNS). In linkage between neurons, balance is modulated by a network of stimulating and blocking circuits. Disruption of both excitatory and inhibitory systems can collapse the balance and cause epileptic discharges. Axonal conduction is one of the two main systems fundamental the electrophysiological excitability and communication substantiates through action potentials and cell-to-cell signal transmission through synaptic transmission. Axonal conduction of action potentials is the fundamental mechanism for the cell-to-cell communication through synaptic transmission[31].

SE is a neurological emergency identified with recurrent seizures that last longer than 30 minutes without returning to the central functions of the central nervous system. This definition may include

having two or more seizures in which seizures are not fully achieved[32]. During SE seizures, as a result of changes in the function and/or synthesis of ion channels induce acute changes in neuron excitability during seizures. This leads to increase in dendritic stimulation and a greater postsynaptic potential stimulus[33]. Since cells with epilepsy that cause gliosis, damage their ability to buffer extracellular K^+ ions cause an increase in K^+ ions outside the cell, leading to a lowered excitability threshold and epilepsy seizures. In addition, extracellular K^+ ion concentration increases in epileptogenic regions due to the decrease in Na^+-K^+/ATPase activity. In this way, the neurons will be stimulated and give arise occurance of discharges and spread more easily[34].

In another study, the expression of voltage-gated potassium channel 4.2 (Kv4.2) and voltage-gated potassium channel 4-interacting protein (KChIP1) in rats formed with Li-pilocarpine were investigated. It was reported that 1 day after SE, Kv4.2 expression increased in both the hippocampus and cortex and KChIP1 expression increased in the hippocampus[35]. These results appear to be compatible with our results (Fig 1, Fig 2).

KCNQ3 is constantly expressed in neuronal cells in the brain. The highest expression levels of KCNQ3 have been reported to be noted in the cerebral cortex, thalamus, hippocampus and caudate / putamen. Some nuclei of the amygdala and the hypothalamus have been found to show KCNQ3 expression[36]. KCNQ2 and KCNQ3 were determined by homology to KCNQ1, as well as positional cloning in families with benign familial neonatal convulsions (BFNC), a form of neonatal epilepsy. These are mainly expressed in neuronal tissue, involving sympathetic ganglia, and the expression patterns in the brain overlap substantially. Similar to KCNQ1, both KCNQ2-3 give slowly activated K^+ currents in depolarization[37,38].

Surprisingly, however, studies on which potassium ion channel variants can be functionally classified by electrophysiological and pharmacological approaches are limited and rather inconsistent. It can be assumed that such differences are related to variety distict operations and quantification procedures. Notwithstanding, systematic research on the comparative significance of the components for channel expression and role in the animal brain is rare in the literature and transformation studies comparing informations from neonatal and adult brain with human details are sufficient. In epilepsy, determining the molecular mechanisms and genetic factors that cause disease, understanding the physiopathology, and developing new treatment proposals in the light of the defined details are important in terms of revealing drug resistance mechanisms, especially in drug-resistant epilepsies. In epilepsy, determining the molecular mechanisms and genetic factors are important in terms of revealing drug resistance mechanisms[39].

Considering that the majority of antiepileptics act directly or indirectly through ion channels or receptors, a treatment approach becomes even more important. Today, with the classical antiepileptic treatment approach, the genetic mutation that can be detected in the patient is also taken into account[40].

As a result, the discovery of K^+ ion channels that encode genes that affect susceptibility and disease progression will provide insight into the molecular events of epileptogenesis, improve molecular diagnostic use, and set new therapeutic targets for the treatment of human epilepsy[41]. Understanding the mechanism of potassium ion channels functioning to cause epilepsy will give new insights into the internal work of neural circuits and help develop new therapies. The pathophysiology of epileptic seizures is not fully known. Also, the same pathophysiology is not valid in all epileptic seizures. Anticonvulsants block voltage sensitive Na^+ ion channels, reducing the duration of epileptic discharges and action potentials that occur with each discharge[42].

The molecular mechanisms regulating K^+ ion channels are largely unknown. There is a need for future preclinical experimental studies to fully understand the events in the voltage dependent channels during epilepsy and to solve the mechanisms of the drugs used in the treatment through these channels. Our study is a preliminary study which is a preliminary study examining the changes in gene expression levels of K^+ ion channels in the experimental animal model performed with status epilepticus with Li-pilocarpine. In future studies, experimental studies are planned on the changes in K^+ ion channels for treatment by creating new experimental models with convulsive drugs. The main limitations of the study are that there are no similar studies in the literature and that the expression level changes of KCNQ1 and KCNQ3 genes in the epileptic model are not known. A more detailed examination of K^+ ion channels will shed light on the elimination of effective mechanisms during seizure and treatment in epilepsy. In order to obtain more

detailed findings, the correlation between ion channels can be evaluated by examining the changes in other ion channels in the SE model. We believe that our study will be the basis for further studies in this field.

In conclusion, the datas obtained in the experimental animal model in our study will enable us to understand the mechanisms by which specific channelopathies contribute to epilepsy and will lead to advances in the treatment strategies of the disease. Although the disorder of K^+ ion channels is associated with many neurological illness, its role is not fully understood. Changes in the expression of K^+ ion channels will help to understand its role in the pathogenesis of epilepsy. Regulation of K^+ ion channel expression is important for the development of drugs and treatments. Understanding the function of K^+ ion channel expression in experimental and clinical studies will contribute to the development of appropriate therapeutic strategies in epileptic diseases.

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