Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Expression of cardiac inwardly rectifying potassium channels in pentylenetetrazole kindling model of epilepsy in rats

Enes Akyüz[1*], Pinar Mega Tiber[2], Merve Beker[3], Fahri Akbaş[3]

[1] Yozgat Bozok University, Medical Faculty, Department of Biophysics, Erdoğan Akdağ Yerleşkesi, 66100, Yozgat, Turkey
[2] Marmara University, Medical Faculty, Department of Biophysics, Başıbüyük Mah. Maltepe Başıbüyük Yolu Sok. No:9/1. Maltepe, Istanbul, Turkey
[3] Bezmialem Vakif University, Medical Faculty, Department of Medical Biology, Adnan Menderes Bulvarı Vatan Caddesi 34093 Fatih, Istanbul, Turkey

Correspondence to: enesakyuz25@gmail.com

Received May 22, 2018; **Accepted** December 17, 2018; **Published** December 31, 2018

Doi: http://dx.doi.org/10.14715/cmb/2017.64.15.8

Abstract: Clinical and experimental studies show that epilepsy affects cardiac function; however, the underlying molecular mechanism has not been fully elucidated. Inwardly-rectifying potassium (Kir) channels transport K^+ ions into excitable cells such as neurons and cardiomyocytes; they control the cell excitability by acting towards the repolarization phase of cardiac action potential. Kir channel expression has been previously shown to vary in epileptic brains, at the same time seizures are known to affect the autonomic nervous system. Kir channel expression in cardiac tissue is a possible mechanism for the explanation of cardiac pathology in epilepsy. We investigate the expression of Kir channels in epileptic cardiac tissue by using pentylenetetrazole (PTZ)-kindling model in rats. Our molecular analyses showed significant decrease in cardiac Kir channel mRNA and protein expression of PTZ-kindled rats. Interestingly, both Kir2.x, which directs I_{K1} flux in ventricular tissue and Kir3.x, which is responsible for I_{KACh} in the atria, were observed to decrease significantly. Kir channel expression also differs between females and males. This is the first study to our knowledge in epileptic cardiac tissue showing the expression of Kir channels. Our results show that Kir channels may play a role in cardiac pathology associated with epilepsy.

Key words: Epilepsy; Kir channels; Heart; Rats; Protein and gene expression.

Introduction

Epilepsy is a chronic neurological disorder characterized by neuronal discharges resulting from impairment of excitatory and inhibitory balance, caused by functional and structural changes in the brain and the risk of sudden unexpected death is 24 times higher than the normal population (1-3). Approximately 2% of the world's population is known to have epilepsy (4); besides the important advances in treatment lately, the reason for high rates of drug resistance is not known (5). Sudden unexpected death in epilepsy (SUDEP) is a leading cause of mortality among drug-resistant young-adult cases and one or more cardiac function anomalies in around 40% of the patients have been reported (6, 7). Additionally, heart rate changes are an important sign of autonomic function anomalies and there are various clinical epilepsy studies emphasizing autonomic function alterations (8, 9). It is also shown that tonic-clonic seizures, a type of generalized seizures, could contribute to development of cardiac arrhythmias by affecting autonomic function during ictal, postictal and interictal periods, being an important approach for the SUDEP mechanism (10, 11). Previous studies have not been able to show a clear connection between the molecular basis of SUDEP and the specific risk factors and pathophysiology of the condition (12).

Inwardly-rectifying potassium (Kir) channels play key roles in cellular excitability of both neurons and cardiomyocytes, towards stabilization of resting membrane potential (13). Kir channels are also prominent in epilepsy pathology are suggested as one of the most important candidates for cardiac channelopathy in epilepsy. These channels are found in tetrameric structures each containing two trans-membrane domains and seven different subunits (Kir1-Kir7). They perform their physiological tasks by acting like a diode; carrying K^+ ions into the cell, where potentials are more negative than the potassium equilibrium potential (14). In recent studies, cerebral Kir channel function and expression (especially Kir2.x, Kir3.x, Kir4.1 and Kir6.2) has been identified by studies involving human epilepsy and animal models (15). Additionally, important role of Kir2.x, Kir3.x and Kir6.x channels in normal functioning of the heart has been supported by $Kir^{(-/-)}$ knockout mouse and clinical studies (16).

Kir2.x (classic Kir channels), possessing a strong inward rectifying characteristic, is responsible for major potassium currents (I_{K1}) in cardiomyocytes and the most abundant channels in ventricles (17). Also, all classic Kir channels playing an important role in determination of resting potential and neuronal excitability are expressed in soma and dendrites of neurons (18). Kir3.1 and Kir3.4 channels of G-protein gated Kir3.x family

that are prominently found in atrial tissue regulate I_{KACh} current, that is activated by adenosine acetylcholine released from vagal nerve and and also play a role in reducing heart rate in addition to activity of other Kir channels. Kir3.x channels located on dendritic spines in various regions of the brain are involved in production of slow inhibitory postsynaptic potential (19). Kir4.1 channels, possessing a central role in epilepsy, are medium-grade inwardly rectifying channels expressed in heart and central nervous system glial cells, and when combined with Kir5.1 forming a heteromeric structure present strong inward rectifying (20). Kir6.x (ATP-sensitive K^+ channels, K_{ATP}) are found, intensively, in cardiac tissue sarcolemma membranes and present a "weaker" inward-rectification tendency compared to classic Kir channels. The opening of these channels are blocked by internal ATP, however, in conditions such as ischemia they open with a protective role by shortening the action potential (21). Neuronal K_{ATP} channels correlate the metabolic status of neurons to their excitability by detecting changes of intracellular phosphate potential (e.g. ATP/ADP ratio) (22).

Expression of Kir channel coding genes (*KCNJ* subfamily) were defined by investigations on rats and human cell studies (23, 24). Accordingly, human Kir genes causing changes in Kir channel expression and/or biophysical properties, and five different diseases with various cardiac arrhythmias have been identified (25).

Even though importance of Kir channels in normal cardiac function and expressional changes in epileptic brain have been previously reported but their function in cardiac pathology of epilepsy was unknown. In this study, we have investigated cardiac Kir channel protein and gene expression by implementation of PTZ-kindling model mimicking epilepsy generated tonic-clonic seizures in rats and obtained significant changes in expressions of these channels. We also show electrocardiographic recordings and blood pressure changes during epileptic seizures. Expressional changes of Kir channels show that they might vary during epileptic cardio-pathology. We propose a novel possible mechanism for cardiac dysfunction and SUDEP.

Materials and Methods

Ethics statement

Animals were treated according to the NIH Animal Care and Use Guide. All procedures performed on animals were approved by the Animal Ethics Committee of the Bezmialem Vakif University (ethics committee decision number: 2016/111). Furthermore, all operations and procedures were performed under anesthesia induced by ketamine and xylazine. Great efforts were made to minimize the pain.

Animals

The experiment was performed using 280-380 g male/female Wistar albino rats obtained from the Research Center of Bezmialem Vakif University. The rats were placed in cages at controlled temperature (24 °C ± 2 °C), free access to water and food, and kept in a 12-hour light-dark cycle. The animals were randomly divided into two groups; all male/female PTZ kindling epilepsy groups and their controls contained 10 rats (n=40). The animals were administered 35 mg/kg of PTZ dissolved in 0.9% NaCl, and injected with 0.09% saline on the control groups.

Pentylenetetrazole (PTZ) kindling epilepsy model

PTZ (P6500, Sigma, St. Louis, MO, USA) - a GABAA receptor antagonist - was dissolved in 0.9% saline and administered intraperitoneally at a dose of 35 mg/kg. Injections were administered three times (Monday, Wednesday and Friday) per week for one month to load the PTZ kindling on the rat and behaviors of them were observed for 30 minutes individually to note according to the following system in terms of epileptic seizure scoring (26).

- Phase 0: No response
- Phase 1: Ear and facial twitching
- Phase 2: Myoclonic body jerks
- Phase 3: Clonic forelimb convulsions
- Phase 4: Tonic – clonic seizures
- Phase 5: Generalized tonic – clonic seizures.

Rats were given a challenging PTZ dose 1 week after the last PTZ injection to assess the sensitivity or to demonstrate improved seizure sensitivity in both female and male epilepsy groups. Animals with phase 4 or 5 seizures were considered to be completely kindled.

Electrocardiographic recording (ECG) and blood pressure measurement

Recordings were taken from all animals using the LabChart 7 (ADInstruments Ltd, Oxford, UK) to show the autonomic nervous system changes in epileptic rats. ECG recordings were measured by placing fine-tipped electrodes in the forelimbs and left flank of the rats. Blood pressure measurements were performed with the jugular vein and the right carotid artery being presented for saline administration and recording of the pressure, respectively. Tracheostomy provided mechanical ventilation.

Tissue preparation process

At the end of the experiment, rats were sacrificed by decapitation followed by removal of the heart under aseptic conditions, immediately. The atrium and ventricular regions were micro-dissected from sections of heart on ice and immediately stored at -80 °C until use.

Real-time polymerase chain reaction (PCR)

The heart tissues from rats were dissected, rapidly frozen in liquid nitrogen, and stored until use at -80 °C. Total RNA was isolated from the frozen tissues using High Pure RNA Isolation Kit (#11828665001, Roche) with Trizol Reagent according to manufacturer's protocol. RNA was quantified using a Nanodrop ND-1000 (Thermo Fischer Scientific).

cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems #4368814) for quantitative real time-PCR according to the manufacturer's protocol. The related genes were detected using SYBR Green PCR kit (iTaq™ Universal SYBR® Green Biorad, 10032048) in real-time fluorescent quantitative PCR analyzer (cfx Biorad, USA) in condition as follows: 95 °C, 30 s; 95 °C, 5 s; 60 °C, 30 s and 40 cycles. The relative expression levels of inwardly rectifier potassium channels (Kir channels; Kir2.1,

Table 1. Primers of Kir channels for quantitative real-time polymerase chain reaction method.

Genes	Forward primer (5' – 3')	Reverse primer (5' – 3')
KCNJ2	GCAAACTCTGCTTGATGTGG	TCATACAAAGGGCTGTCTTCG
KCNJ12	TTCTCTCTGGCCTTTCTTGC	CTTCAGTCACGCATCGTAGC
KCNJ4	CTACCGCAGGGAATGAGCCAT	TCAGATGGCTCATTCCCTGCG
KCNJ3	CTGACCGCTTCACATAGC	CTCCAGACTGGGATAGAC
KCNJ5	GCTGGCGATTCTAGGAATGC	TCTGTGGCAATGGGGACATAA
KCNJ11	CCTACACCAGGTGGACATCC	CAGGCTGCGGTCCTCATCAA
KCNJ10	AGTAGACACAGCCTCTGATAGCC	GCAGGTGTGAACTCGTAGCC

Kir2.2, Kir2.3, Kir3.1, Kir3.4, Kir4.1 and Kir6.2) were calculated from Ct values using 2-ΔΔt method. The primer sequences for each gene were shown in Table 1.

A two-step PCR was performed using Chromo 4 real-time PCR analysis system with Opticon Monitor Version 3.1 (BioRad). Denaturation was at 95 °C for 15 s followed by annealing and extension at 60 °C for 60 s for 40 cycles, 50 °C for 2 min, and 95 °C for a 10-min pre-treatment. All primer/probe sets were purchased from Applied Biosystems: GFAP; Kir2.1 (*KCNJ2*), Kir2.2 (*KCNJ12*), Kir2.3 (*KCNJ4*), Kir3.1 (*KCNJ3*), Kir3.4 (*KCNJ5*), Kir4.1 (*KCNJ10*), Kir6.2 (*KCNJ11*) and GAPDH. GAPDH was used as an internal control gene. Data obtained from 3 to 4 hearts for each column are presented as the fold-change of control mean ± SEM.

Protein isolation and sample preparation

Frozen cardiac tissue samples were weighed and treated with lysis buffer (RIPA buffer, Cell Signaling; 9806), containing protease and phosphatase inhibitor cocktail (Cell Signaling; 5871, 5870), proportional to sample weights. Tissue samples were homogenized and left for incubation to better observe the effects of buffer solution. Protein concentration was measured spectrophotometrically by bicinchoninic acid method (BCA assay, Thermo Scientific; 23225). Samples were used according to calculations for 20 µg protein per well compared to their concentration. Samples were mixed with Laemmle buffer (LB, Biorad, 1610-737) with a ratio of 1:1 and heated at 95 ºC for 5 minutes to allow denaturation of the proteins and denatured samples are stored at -20 ºC for short-term.

Western blotting

Frozen heart samples were homogenized with Lysis buffer containing protease inhibitor cocktail (20-188, Millipore; SC-29130, Santa Cruz) and proteins were isolated. Following concentration measurements of proteins, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied. Afterwards, proteins were transferred to polyvinyl fluoride (PVDF) membranes and incubated with rat monoclonal Kir2.1 (ab65796, ABCAM), Kir2.2 (ab84821, ABCAM), Kir2.3 (APC-032, Alomon Lab.), Kir3.1 (APC-005, Alomon Lab.), Kir3.4 (APC-027, Alomon Lab.), Kir 4.1 (ab105102, ABCAM) and Kir6.2 (APC-020, Alomon Lab.) antibodies that were detected by chemiluminescence labeling. Protein loading was controlled using actin antibody. Protein abundance was evaluated by densitometry. All animals in each four groups were analyzed separately. For these blots of animals, mean values were calculated and statistically analyzed by one-way ANOVA.

Statistical analysis

Mean and standard deviation values were calculated for the data, followed by one-way analysis of variance (ANOVA), Mann-Whitney-U test statistical analyses, using the SPSS software. p values at 0.05 were treated as statistically significant.

Results

Development of kindled seizures

Chronic epileptic seizures were produced by kindling in male and female rats that were administered sub-convulsive 35 mg/kg PTZ three times per week for 1 month (Figure 1). Generalized tonic-clonic seizures, the last phase of the Racine's scaling system were observed almost in all animals. As shown in Figure 1, phase 5 (generalized tonic-clonic) seizures were seen in all female PTZ-kindled rats by the end of 13th injection, however, the mean scale remained around 4.33 (± 0.62) in male rats. Twelve animals were included in epilepsy groups; however, 2 rats were excluded from both groups due to early death and/or insufficient epileptic phase (rats with a score under phase 3). Weights of the rats were measured regularly during PTZ injection process and no significant change was observed (data not shown).

Change of autonomic nervous system in PTZ-epilepsy model by ECG recording and blood pressure measurement

To demonstrate that the autonomic nervous system changes in epilepsy, ECG and blood pressure recordings were taken simultaneously from the rats. It was shown that the autonomic nervous system of the PTZ-epilepsy model changes obtained significantly (*p<0.05).

During the epileptic seizure, the animals in which the ECG electrodes were attached had excessive move-

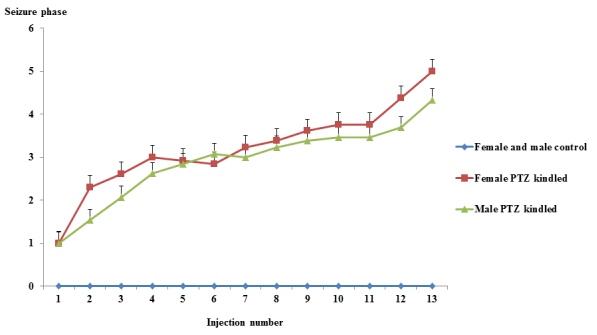

Figure 1. Epileptic seizure phases are accepted in terms of Racine's scaling system (26). Severity of convulsions induced by pentylenetetrazole (PTZ) in rat intraperitoneally. PTZ kindled epileptic male and female rats have reached the maximum level of seizure (mean±SEM) at the last injection.

ment of the upper and lower extremities, the recording during the attack was very noisy and was not suitable for analysis. Therefore, analyzes of R-R, QRS, ST, P, QT, distances were performed based on 30 minutes of stable ECG recordings from records at pre-ictal and post-ictal periods. Table 2 shows the ECG results of female rats. As a result, there was a significant decrease in RR, QT and ST segments in female PTZ-epilepsy group compared to female control group (*p <0.05).

ECG findings of male rats are shown in Table 3. A significant decrease in ST interval in male PTZ-epilepsy group rats compared to male control group was observed (*p<0.05). Other parameters were not changed.

The blood pressure measurements from the PTZ-epilepsy and control group animals were taken from the carotid artery. Control group blood pressure of rats and PTZ-epilepsy group rats were compared according to blood pressure changes during ictal period. As a result of the measurements, blood pressures decreased significantly from 121.3±39.7 mmHg to 64.60±9.2 mmHg in the female PTZ group after 8.8 (± 1.3) s from the onset of epileptic seizures. The blood pressure values returned to normal values in the post-ictal period.

PTZ-kindling model epilepsy on the rat exhibit altered cardiac Kir mRNA and protein expression

We have analyzed Kir mRNA and protein expression in cardiac tissue with the purpose of investigating a possible molecular mechanism explaining previously observed cardiac function alterations in epilepsy. Kir channel expression investigations were performed in either ventricular or atrial tissues based on discrimination according to the regions that each channel is found the most intensely. Accordingly, expressions of classic Kir channels (Kir2.1, Kir2.2 and Kir2.3), Kir4.1 and Kir6.2 were evaluated in ventricles, while Kir3.1 and Kir3.4 channel expressions were evaluated in atria.

mRNA expression

In female PTZ-kindled rats, Kir2.1 channel mRNA levels were significantly decreased by 4.94 folds (Figure 2A, *p<0.05), Kir2.2 channel was also significantly decreased by 5.5 folds, (Figure 2B, ***p<0.001); while no significant change was observed in Kir2.3 channel expression (Figure 2C, p>0.05). In male PTZ-kindled rats, no significant change was observed in Kir2.1 and Kir2.3 channel mRNA expression levels (Figure 2A; 2C, p>0.05), while Kir2.2 channel mRNA expression was decreased by 2.28 folds (Figure 2B, *p<0.05). Ventricular Kir4.1 and Kir6.2 channel mRNA levels were significantly decreased by 5.05 and 6.75 folds in female rats, respectively, (Figure 2F, ***p<0.001; Figure 2G, **p<0.01); while Kir4.1 alone was decreased by 3.63 folds in male rats (Figure 2F, **p<0.01). Investigations performed on atrial tissue revealed that Kir3.1 channel mRNA levels were significantly decreased in both female and male PTZ-kindled groups (2.55 and 2.93 folds, respectively) (Figure 2D, **p<0.01). Significant decrease in Kir3.4 channel mRNA levels were observed only in female PTZ-kindled rats (2.37 folds) (Figure 2E, *p<0.05).

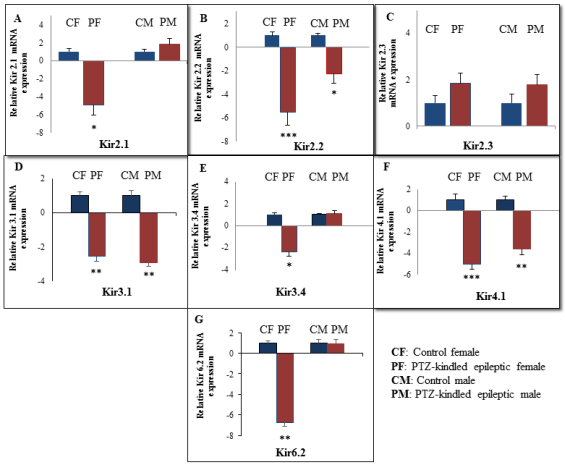

Figure 2. Relative mRNA expressions of Kir2.1, Kir2.2, Kir3.1, Kir3.4, Kir4.1, Kir6.2 channels in PTZ kindled epileptic female rats; Kir2.2, Kir3.1 and Kir4.1 in PTZ kindled epileptic male rats are significantly decreased. CF: control female rats (n=10); PF: PTZ kindled epileptic female rats (n=10); CM: control male rats (n=10); PM: PTZ kindled epileptic male rats (n=10). Kir2.1, Kir2.2, Kir2.3, Kir4.1 and Kir6.2 channels were investigated in ventricle tissue; Kir3.1 and Kir3.4 in atrium. A) Relative mRNA expression of Kir2.1 for female and male rats (among control and PTZ-kindled epilepsy; B) Kir2.2; C) Kir2.3; D) Kir3.1; E) Kir3.4; F) Kir4.1 and G) Kir6.2. The grouping of gels/blots cropped from different parts of the same gel. *p<0.05; **p<0.01; and ***p<0.001 by one way Mann-Whitney U test.

Table 2. ECG recording of female rats.

Intervals	Control group (ms)	PTZ epilepsy group (ms)	p value
R-R*	**338,1 (±31)**	**294,1 (±30,1)**	**0,009**
QRS	16,3 (±2,4)	13,4 (±3,7)	0,068
QT*	**65,5 (±6,3)**	**58,6 (±7,5)**	**0,034**
ST*	**51,1 (±8,5)**	**42,7 (±4,8)**	**0,034**
P	21,8 (±1,1)	21,1 (±1,2)	0,315

*p<0.05.

Table 3. ECG recording of male rats.

Intervals	Control group (ms)	PTZ epilepsy group (ms)	p value
R-R	282,2 (±15,2)	312,3 (±31,2)	0,082
QRS	14,1 (±1,7)	12,9 (±2,4)	0,429
QT	59,3 (±2,6)	53,5 (±8,7)	0,429
ST*	**46,6 (±3,4)**	**40,0 (±8,1)**	**0,049**
P	21,2 (±0,4)	22,3 (±1,2)	0,082

*p<0.05.

Protein expression

Cardiac Kir channel protein quantity was evaluated by Western-blotting to determine if significantly reduced mRNA levels translated to a decrease in protein.

Classic Kir channels were evaluated primarily in investigations performed on samples obtained from ventricular tissue. Kir2.1 channel protein expression was significantly decreased by 50% in female PTZ-kindled rats (Figure 3A, **p<0.01), while no significant change was observed in male rats. Significant decreases were observed in Kir2.2 channel expression in both female and male rats (%55 (***p<0.001) and % 30 (**p<0.01), respectively, Figure 3B). Kir2.3 channel expression was significantly changed only in female PTZ-kindled rats (decrease by 48% Figure 3C; ***p<0.001). In investigations performed on atrial tissue samples, Kir3.1 channel protein expression was significantly decreased by

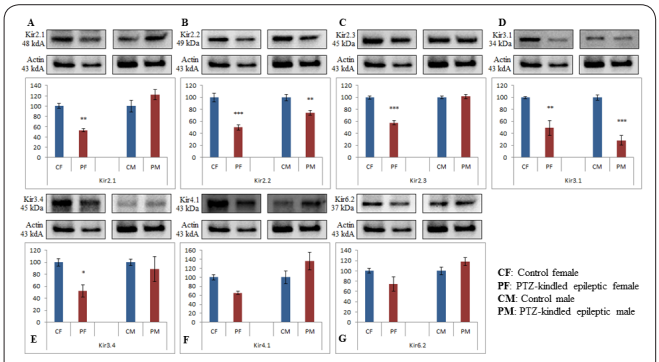

Figure 3. Protein expressions of Kir2.1, Kir2.2, Kir2.3, Kir3.1, Kir3.4 channels in PTZ kindled epileptic female rats; Kir2.2, Kir3.1 in PTZ kindled epileptic male rats are significantly decreased. CF: control female rats (n=10); PF: PTZ kindled epileptic female rats (n=10); CM: control male rats (n=10); PM: PTZ kindled epileptic male rats (n=10). Kir2.1, Kir2.2, Kir2.3, Kir4.1 and Kir6.2 channels were investigated in ventricle tissue; Kir3.1 and Kir3.4 in atrium. A) Protein expression of Kir2.1 for female and male rats (among control and PTZ-kindled epilepsy; B) Kir2.2; C) Kir2.3; D) Kir3.1; E) Kir3.4; F) Kir4.1 and G) Kir6.2. Representative Western blot of Kir channels have been shown above the each graphics. *p<0.05; **p<0.01; and ***p<0.001 by one way Mann-Whitney U test.

55% in PTZ-kindled female rats and 78% in PTZ-kindled male rats (Figure 3D, **p<0.01 and ***p<0.001). Kir3.4 channel protein expression was significantly decreased by 50% in female PTZ-kindled rats (*p<0.05), while no significant change was observed in male rats (Figure 3E). No significant changes were observed in expression of Kir4.1 and Kir6.2 channels, the two other channels investigated in ventricular tissue, in both female and male PTZ-kindled groups (Figure 3F-3G, p>0.05). These changes were confirmed with three repetitions comparing PTZ-kindled versus control groups.

Discussion

Epileptic seizures causing involvement of central autonomic control centers could alter cardiac functions by exhibiting important effects on autonomic nervous system (27). Additionally, autonomic involvement of cardiovascular and respiratory systems are considered to contribute in development of sudden unexpected death in epilepsy (SUDEP) concept (28). Changes in function and/or expression of ion channels that control cellular excitability of both brain and heart are a strong candidate for cardiac dysfunction mechanism in SUDEP and epilepsy.

As a first finding in our studying, significant decrease in blood pressure and electrocardiography (ECG) recordings have been observed to demonstrate that the autonomic nervous system changes. Blood pressure changes in the ictal period of epileptic rats compared to the control group and also shortening of the ST interval in ECG recordings indicated that the quantities and / or functions of the ion channels found in the heart tissue may altered in epilepsy as secondarily.

In a study published in 2009, mice carrying *KCNQ1* mutation were observed to suffer from both cardiac long QT syndrome that leads to fatal cardiac arrhythmias and ambiguous seizures with unknown origin, therefore, brain-heart potassium channel gene *KCNQ1* was defined as the first ion channel gene that could be responsible for SUDEP (29). In the study conducted by Glasscock *et al.* next year, deficiency of *KCNA1* gene that encodes Kv1.1 potassium channel was shown to cause severe seizures and premature death in mice and this dysfunction was suggested as a possible mechanism for SUDEP by impairing neural control of cardiac rhythmcity (30). Powell *et al.* showed in a recent study, performed with use of experimental animal models, that chronic epilepsy causes the development of a secondary cardiac channelopathy along with electrophysiological changes and this could be a potential explanation for mechanisms underlying cardiac dysfunction in chronic epilepsy patients (31). Accordingly, PTZ-kindling epilepsy model, previously recognized in the literature, was applied to female and male rats to produce tonic-clonic seizures (Figure 1); and a channel family among potassium channels, expressed in both brain and heart, that had been previously defined to have a role in epilepsy however not investigated in cardiac tissue was targeted.

Changes in the expression of cardiac K^+ channels that regulate resting membrane potential close to potassium equilibrium potential (E_K) affects sustaining of action potential, and mostly lead to disruption of cardiac action potential phases or involvement of cardiac rhythmicity (32). Each subtype of inwardly-rectifying potassium channels (Kir channels), expressed on cardiac sarcolemma with a role in controlling of action potential duration, are channels that possess different kinetics and regulations with roles at various stages of action potential (33).

I_{K1} current, directed by powerful Kir channels (Kir2.x, Kir2.1, Kir2.2 and Kir2.3) that are expressed 10 times higher in ventricular tissue compared to atria, plays a role in development of plateau phase of action potential and induces the last phase of rapid repolarization (34, 35). Lopatin *et al.* repressed I_{K1} current by producing transgenic mice expressing a dominant-negative subunit of Kir2.1 channel and observed, by patch clamping, that I_{K1} current was decreased by 95% and action potential was significantly prolonged. In the same study, it was also observed that QRS complex and QT interval were prolonged in surface ECG records of the mice (36). Additionally, temporal lobe epilepsy model in rats was used in another study to investigate the role of Kir channels in epilepsy. It was suggested that upregulation of Kir2.x channels, buffering extracellular K^+, in dentate granule neurons could balance excitation by developing a mechanism against hyperexcitability and this mechanism could present a functional "anti-convulsive" effect (37). In the study investigating hippocampus of pilocarpine temporal lobe epilepsy model rats, significant decrease in Kir2.3 channel mRNA and protein expressions was shown, and delay of epileptiform activity with administration of an agent called tenidap to epileptic rats was observed (38). In clinical studies, mutations seen in *KCNJ2* gene encoding Kir2.1channel were detected to alter channel functions in various epileptic seizure or cardiac symptom related syndromes. Ventricular arrhythmias accompanied by Kir2.1 channel dysfunction, presented with U wave prolongation on ECG are seen in Andersen-Tawil syndrome (39), while it was shown that gain of function due to mutation in channel coding gene in short QT syndrome increases sudden cardiac death

risk (40). In other classic Kir channels, *KCNJ12* and *KCNJ4* coding Kir2.2 and Kir2.3, respectively, no mutations were reported related to epilepsy or cardiac syndromes. Various significant down-regulation of Kir2.x channel mRNA and protein expressions (Figure 2A-C; Figure 3A-C) in cardiac ventricles of PTZ-kindled epileptic rats could be preliminary findings pointing that these channels, directing the most powerful current in heart, could cause secondary cardiac problems in epilepsy. Especially the findings of prolonged QT interval in Kir2.1 channel absence (34) and down-regulation of Kir2.1 findings obtained in our study emphasize that this channel family must be comprehensively investigated in epileptic cardiac myocytes (patch clamp), defining its function in action potential, and its possible role in SUDEP mechanism must be discussed.

In addition to Kir2.x channels that direct I_{K1} current with a classic powerful inward rectifying characteristic, Kir3.1 and Kir3.4 channels directing I_{KACh} current are also present, with a role in cardiac major potassium current, and contributing to cardiac excitability. These channels regulate heart-rate by becoming activated by acetylcholine released from vagal nerve (13, 17). They are determined as a possible target in atrial fibrillation, one of the most common cardiac arrhythmias seen in clinic (41). Additionally, it was reported in a clinical case study that a mutation (Gly387Arg) seen in *KCNJ5* gene coding Kir3.4 channel leads to a decrease in I_{KACh} current due to channel's loss-of-function and this finding could play a role in long QT syndrome (42). Gain of function in Kir3.x channels in nervous system was reported to decrease neuronal activity and its loss-of-function was reported to lead to excessive neuronal excitability and epilepsy (22). In a study by Mazarati *et al.*, Kir3.x channel inhibition induced by intrathecal tertiapin administered was shown to be pro-convulsive; in addition, excitation of galanin type 2 receptors activating Kir3.x channels was reported to prevent epileptogenesis kindled in rats (43). In another study, Kir3.x activator agent named ML297 was shown to exhibit treating effects in two different rat epilepsy models, and Kir3.x channels were determined as a new target in epilepsy treatment (44). Observation of treating findings of Kir3.1 and Kir3.4 channel activations necessitates investigating expressions of these channels in different regions of the brain. In addition, significant down-regulations observed in Kir3.1 and Kir3.4 channel mRNA and protein expressions in atrial tissue of PTZ-kindled rats (Figure 2D-E; Figure 3D-E, respectively) could be the sign of impaired functioning of these channels, with a failure of heart-rate regulation role, leading to rhythmic anomalies in cardiac tissue during seizures simultaneously. Function of Kir3.1 and Kir3.4 channels in atrium could be determined with new studies through transgenic animals in case of possible down-regulation/loss of function in epilepsy.

It was reported in clinical and experimental studies that Kir4.1 channels found most commonly in oligodendrocytes producing myelin sheath, and astrocytes enclosing neurons, possesses a central role in epilepsy due to its extracellular K^+ ion buffering property (45). Recently, parenchymal albumin deposition in astrocytes was reported to cause acceleration of epileptiform activity by inducing down-regulation of Kir4.1 channel and increase in levels of extracellular potassium that triggers depolarization. Accordingly, possible importance of effects of depolarization spread on respiratory control, shutting down cardio-respiratory centers and functional dysregulation of neuronal, glial and microvascular networks in blood-brain barrier in SUDEP pathophysiology is discussed (46). Kir6.2, is responsible for K^+ ion transport during cellular metabolic activities by contributing to electrical signalization in brain and heart tissue, performs the task of balancing against serum glucose concentration (47). However, role of Kir6.2 channels in epilepsy pathophysiology is not known, and it is predicted that a more stimulator environment is present due to the weakening of K_{ATP} channels by high extracellular glucose and intracellular ATP levels (15). In present study, the decrease in epileptic rat cardiac tissue Kir4.1 and Kir6.2 channel mRNA levels are thought to be transcriptional as no significant change in protein expressions of these channel were observed (Figure 2F/3F; Figure 2G/3G, respectively).

Profound ion channels that are present in both brain and heart, shown to exhibit alterations in epilepsy and could be responsible for development of cardiac arrhythmia due to chronic epilepsy have been investigated previously. Additionally, cortical autonomic function impairment was also suggested to play a role; seizure activity was considered to affect the cardiac function by altering autonomic output centered to heart (48, 49). It is well-known that ion channels may differ because of gender (50). The different results of the obtained protein and mRNA expression evaluations in male and female rats may due to the presence of different amounts of Kir channels in females and males (51). In addition, the amount of estrogen in female rats may be thought to be an effect on the expression of Kir channels (52). Accordingly, changes in Kir channel expression may also be present in people with epilepsy; may cause cardiac dysfunction, as an important problem in epileptic patients, or potentially one of the contributing factors to SUDEP.

In present study, we have suggested a new channel family, including the channels directing the main potassium current in the heart (Kir2.x) and opening by spread of neurotransmitters carried by vagal nerve acting as a "bridge" between brain and heart (Kir3.x), as a possible mechanism for secondary cardiac channelopathy in epilepsy due to the changes observed in their expressions. In conclusion, these changes seen in Kir channel expressions could be present in epilepsy patients, leading to cardiac dysfunction, a major problem in epilepsy; or could be one of the factors potentially contributing to SUDEP.

Acknowledgments

We wish to thank Hifa Gulru Caglar and Cagla Yildiz for technical assistance. This work was supported by Bezmialem Vakif University, Scientific Research Unit (grant reference number: 3.2016/22).

Competing financial interests

The authors declare no competing financial interests.

Author contributions statement

E.A. and F.A. designed the experiment. E.A and P.M.T

wrote the main manuscript text and E.A./M.B. prepared the figures and tables.

References

1. Ficker DM, So EL, Shen WK, Annegers JF, O'Brien PC, Cascino GD, Belau PG, Population-based study of the incidence of sudden unexplained death in epilepsy. Neurology, 1998; 51(5): 1270-4.
2. Lhatoo SD and Sander JW, Sudden unexpected death in epilepsy. Hong Kong Med J, 2002; 8(5): 354-8.
3. Leestma J.E., Forensic considerations in sudden unexpected death in epilepsy. Epilepsia, 1997; 38(11 Suppl): S63-6.
4. Hesdorffer DC, Logroscino G, Benn EK, Katri N, Cascino G, Hauser WA, Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. Neurology, 2011; 76(1): 23-7.
5. Feely M., Fortnightly review: drug treatment of epilepsy. BMJ, 1999; 318(7176): 106-9.
6. Laxer KD, Trinka E, Hirsch LJ, Cendes F, Langfitt J, Delanty N, Resnick T, Benbadis SR, The consequences of refractory epilepsy and its treatment. Epilepsy Behav, 2014; 37: 59-70.
7. Nei M., Ho RT, and Sperling MR, EKG abnormalities during partial seizures in refractory epilepsy. Epilepsia, 2000; 41(5): 542-8.
8. Tomson T, Ericson M, Ihrman C, Lindblad LE, Heart rate variability in patients with epilepsy. Epilepsy Res, 1998; 30(1): 77-83.
9. Lotufo PA, Valiengo L, Bensenor IM, Brunoni AR, A systematic review and meta-analysis of heart rate variability in epilepsy and antiepileptic drugs. Epilepsia, 2012; 53(2): 272-82.
10. Devinsky O, Effects of Seizures on Autonomic and Cardiovascular Function. Epilepsy Curr, 2004; 4(2): 43-46.
11. Moseley B, Bateman L, Millichap JJ, Wirrell E, Panayiotopoulos CP, Autonomic epileptic seizures, autonomic effects of seizures, and SUDEP. Epilepsy Behav, 2013; 26(3): 375-85.
12. Dlouhy BJ, Gehlbach BK, and Richerson GB, Sudden unexpected death in epilepsy: basic mechanisms and clinical implications for prevention. J Neurol Neurosurg Psychiatry, 2016; 87(4): 402-13.
13. Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y, Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev, 2010; 90(1): 291-366.
14. Bond CT, Pessia M, Xia XM, Lagrutta A, Kavanaugh MP, Adelman JP, Cloning and expression of a family of inward rectifier potassium channels. Receptors Channels, 1994; 2(3): 183-91.
15. Villa C, and Combi R, Potassium Channels and Human Epileptic Phenotypes: An Updated Overview. Front Cell Neurosci, 2016; 10: 81.
16. Pattnaik BR, Asuma MP, Spott R, Piller DA, Genetic defects in the hotspot of inwardly rectifying K(+) (Kir) channels and their metabolic consequences: a review. Mol Genet Metab, 2012; 105(1): 64-72.
17. Anumonwo JM and Lopatin AN, Cardiac strong inward rectifier potassium channels. J Mol Cell Cardiol, 2010; 48(1): 45-54.
18. Day M, Carr DB, Ulrich S, Ilijic E, Tkatch T, Surmeier DJ, Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and Kleak channels. J Neurosci, 2005;25(38): 8776-87.
19. Gonzalez C, Baez-Nieto D, Valencia I, Oyarzun I, Rojas P, Naranjo D, Latorre R, K(+) channels: function-structural overview. Compr Physiol, 2012; 2(3): 2087-149.
20. Brenner R and Wilcox KS, Potassium Channelopathies of Epilepsy, in Jasper's Basic Mechanisms of the Epilepsies, J.L. Noebels, et al., Editors. 2012.
21. Gross GJ and Auchampach JA, Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res, 1992; 70(2): 223-33.
22. D'Adamo MC, Catacuzzeno L, Di Giovanni G, Franciolini F, Pessia M, K(+) channelepsy: progress in the neurobiology of potassium channels and epilepsy. Front Cell Neurosci, 2013; 7: 134.
23. Karschin C and Karschin A, Ontogeny of gene expression of Kir channel subunits in the rat. Mol Cell Neurosci, 1997; 10(3-4): 131-48.
24. D'Avanzo N, Cheng WW, Xia X, Dong L, Savitsky P, Nichols CG, Doyle DA, Expression and purification of recombinant human inward rectifier K+ (KCNJ) channels in Saccharomyces cerevisiae. Protein Expr Purif, 2010; 71(1): 115-21.
25. Humphries ES and Dart C, Neuronal and Cardiovascular Potassium Channels as Therapeutic Drug Targets: Promise and Pitfalls. J Biomol Screen, 2015; 20(9): 1055-73.
26. Racine RJ, Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol, 1972; 32(3): 281-94.
27. Jansen K and Lagae L, Cardiac changes in epilepsy. Seizure, 2010; 19(8): 455-60.
28. Lee J and Devinsky O, The role of autonomic dysfunction in sudden unexplained death in epilepsy patients. Rev Neurol Dis, 2005; 2(2): 61-9.
29. Goldman AM, Glasscock E, Yoo J, Chen TT, Klassen TL, Noebels JL, Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. Sci Transl Med, 2009; 1(2): 2-6.
30. Glasscock E, Yoo JW, Chen TT, Klassen TL, Noebels JL, Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. J Neurosci, 2010; 30(15): 5167-75.
31. Powell KL, Jones NC, Kennard JT, Ng C, Urmaliya V, Lau S, Tran A, Zheng T, Ozturk E, Dezsi G, Megatia I, Delbridge LM, Pinault D, Reid CA, White PJ, O'Brien TJ, HCN channelopathy and cardiac electrophysiologic dysfunction in genetic and acquired rat epilepsy models. Epilepsia, 2014; 55(4): 609-20.
32. Snyders DJ, Structure and function of cardiac potassium channels. Cardiovasc Res, 1999; 42(2): 377-90.
33. Grandi E, Sanguinetti MC, Bartos DC, Bers DM, Chen-Izu Y, Chiamvimonvat N, Colecraft HM, Delisle BP, Heijman J, Navedo MF, Noskov S, Proenza C, Vandenberg JI, Yarov-Yarovoy V, Potassium channels in the heart: structure, function and regulation. J Physiol, 2017; 595(7): 2209-2228.
34. Lopatin AN and Nichols CG, Inward rectifiers in the heart: an update on I(K1). J Mol Cell Cardiol, 2001; 33(4): 625-38.
35. Zaritsky JJ, Eckman DM, Wellman GC, Nelson MT, Schwarz TL, Targeted disruption of Kir2.1 and Kir2.2 genes reveals the essential role of the inwardly rectifying K(+) current in K(+)-mediated vasodilation. Circ Res, 2000; 87(2): 160-6.
36. McLerie M and Lopatin AN, Dominant-negative suppression of I(K1) in the mouse heart leads to altered cardiac excitability. J Mol Cell Cardiol, 2003; 35(4): 367-78.
37. Young CC, Stegen M, Bernard R, Müller M, Bischofberger J, Veh RW, Haas CA, Wolfart J, Upregulation of inward rectifier K+ (Kir2) channels in dentate gyrus granule cells in temporal lobe epilepsy. J Physiol, 2009; 587(Pt 17): 4213-33.
38. Xu L, Hao Y, Wu X, Yu P, Zhu G, Hong Z, Tenidap, an agonist of the inwardly rectifying K+ channel Kir2.3, delays the onset of cortical epileptiform activity in a model of chronic temporal lobe epilepsy. Neurol Res, 2013; 35(6): 561-7.
39. Haruna Y, Kobari A, Makiyama T, Yoshida H, Akao M, Doi T, Tsuji K, Ono S, Nishio Y, Shimuzu W, Inoue T, Murakami T, Tsuboi N, Yamanouchi H, Ushinohama H, Nakamura Y, Yoshinaga M, Horigome H, Aizawa Y, Kita T, Horie M, Genotype-phenotype correlations of KCNJ2 mutations in Japanese patients with Andersen-Tawil syndrome. Hum Mutat, 2007; 28(2): 208.
40. Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dham-

oon A, Napolitano C, Anumonwo J, di Barletta MR, Gudapakkam S, Bosi G, Stramba-Badiale M, Jalife J, A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res, 2005; 96(7): 800-7.

41. Dobrev D, Friedrich A, Voigt T, Jost N, Wettwer E, Christ T, Knaut M, Ravens U, The G protein-gated potassium current I(K,ACh) is constitutively active in patients with chronic atrial fibrillation. Circulation, 2005; 112(24): 3697-706.

42. Yang Y, Yang Y, Liang B, Liu J, Li J, Grunnet M, Olesen SP, Rasmussen HB, Ellinor PT, Gao L, Lin X, Li L, Wang L, Xiao J, Liu Y, Liu Y, Zhang S, Liang D, Peng L, Jespersen T, Cheng YH, Identification of a Kir3.4 mutation in congenital long QT syndrome. Am J Hum Genet, 2010; 86(6): 872-80.

43. Mazarati A, Lundström L, Sollenberg U, Shin D, Langel U, Sankar R, Regulation of kindling epileptogenesis by hippocampal galanin type 1 and type 2 receptors: The effects of subtype-selective agonists and the role of G-protein-mediated signaling. J Pharmacol Exp Ther, 2006; 318(2): 700-8.

44. Kaufmann K, Romaine I, Days E, Pascual C, Malik A, Yang L, Zou B, Du Y, Sliwoski G, Morrison RD, Denton J, Niswender CM, Daniel JS, Sulikowski GA, Xie XS, Lindsley CW, Weaver CD, ML297 (VU0456810), the first potent and selective activator of the GIRK potassium channel, displays antiepileptic properties in mice. ACS Chem Neurosci, 2013; 4(9): 1278-86.

45. Steinhauser C, Grunnet M, and Carmignoto G, Crucial role of astrocytes in temporal lobe epilepsy. Neuroscience, 2016; 323: 157-69.

46. Michalak Z, Obari D, Ellis M, Thom M, Sisodiya SM, Neuropathology of SUDEP: Role of inflammation, blood-brain barrier impairment, and hypoxia. Neurology, 2017; 88(6): 551-561.

47. Haider S, Antcliff JF, Proks P, Sansom MS, Ashcroft FM, Focus on Kir6.2: a key component of the ATP-sensitive potassium channel. J Mol Cell Cardiol, 2005; 38(6): 927-36.

48. Ravindran K, Powell KL, Todaro M, O'Brien TJ, The pathophysiology of cardiac dysfunction in epilepsy. Epilepsy Res, 2016; 127: 19-29.

49. Page T, Rugg-Gunn FJ, Bitemporal seizure spread and its effect on autonomic dysfunction. Epilepsy Behav, 2018; 84: 166-172.

50. Morales MA, Gonzalez-Rodriguez S, Ferrer-Montiel A, TRP Channels as Potential Targets for Sex-Related Differences in Migraine Pain. Front Mol Biosci, 2018; 73(5): 1-12.

51. Rivero A and Curtis AB, Sex differences in arrhythmias. Current Opinion in Cardiology, 2010; 25(1): 8-15.

52. Bhave G, Lonergan D, Chauder BA, Denton JS, Small-molecule modulators of inward rectifier K+ channels: recent advances and future possibilities. Future Med Chem, 2010; 2(5): 757-74.