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INVESTIGATION OF PHYSIOLOGICAL ROLE OF MITOCHONDRIAL K_{ATP} CHANNEL'S ON PENICILLIN G INDUCED EXPERIMENTAL EPILEPSY MODEL IN RATS

ABSTRACT

Epilepsy is one of the neurological diseases that is commonly seen in the world. It is characterized by excessive activation of neurons that can not be controlled by the central nervous system. ATP dependent potassium (K_{ATP}) channel modulation is related with the epilepsy. This study is intended to research the physiological role of mitochondrial KATP channels in epilepsy in electrophysiological perspective. And bepridil has been used for this purpose. Wistar albino rats have been used. Animals have been divided into three main groups; Control, bepridil applied groups in pre-seizure, and during seizure. As a result, bepridil once applied prior to seizure in 0.1 and 1mg/kg doses increased the latency period of the seizure. Bepridil showed anticonvulsant effect at doses of 0.1 and 1 mg/kg before and during seizure groups. Closure of sarcoplasmic channels and opening of mitochondrial channels may be important to decrease the convultion occurred during epilepy.

Keywords: Epilepsy, Electrophysiology, K_{ATP}, Bepridil, Mito K_{ATP} Channel

1. INTRODUCTION

Epilepsy is one of the neurological diseases that has impacted more than 50 million people in the world. There are many identified type of Epilepsy. Among the epilepsy types, 6 out of every 10 cases where the main cause cannot be determined is defined as idiopathic epilepsy. The other one can be identified as symptomatic epilepsy. Possible causes for symptomatic epilepsy are known and they can be listed as brain damage due to prenatal causes, mulfunctions related to congenital abnormalities and genetic conditions, cases, such as brain damage and stokes, where oxygenation of the brain is reduced, infections like meningitis, encephalitis, neurocysticercosis, and brain tumors [1, 2 and 3]. To put it another way, epilepsy can be defined as a disease that arise from shifting of the balance between the excitatory and inhibitory neurotransmitters in the brain in favor of excitation, and it is characterized by concurrent discharging of the neurons. Most of the epilepsy types have a complex genetic etiology involving the interaction of one or more genes and environmental factors. Ion channels located within the cell membrane have effects over the formation of seizures [4].

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K_{ATP} channels exist in brain as well as in many tissues [5 and 6]. They are produced pre- and postsynaptically in the brain. They open or close depending on the intracellular changes in the ratio of ATP/ADP. These channels open once the ATP level decreases and K leaks out of the cell and hyperpolarization occurs [4]. K_{ATP} channels have been initially discovered in the heart [5], and their role has been ultimately understood in the course of the studies related to the secretion of insulin from pancreatic β cells [7 and 8]. It has been confirned via multiple studies that K_{ATP} channels can play an important role in the cellular responses of the tissues to the various metabolic conditions like hyperglycaemia, hypoglycaemia, ischemia, and hypoxia [9]. It has been proven that K_{ATP} channels located in different sections of the brain have differentiated molecular structures and accordingly they possess different functions. Along with the control over synaptic excitability, K_{ATP} channels controls the local K flow as well as they control the local electrical activity [10]. Furthermore, existance of K_{ATP} channels also in nucleus and mitochondria points to the fact that these channels play a critical role in the intracellular metabolic activities [11 and 12]. While a significant number of studies have been conducted to show the effect of other channels on epilepsy, studies regarding the importance of K_{ATP} channels in controlling the seizure threshold are insufficient.

2. RESEARCH SIGNIFICANCE

In this study performed by means of bepridil, we have targetted searching of the physiological role of the closing of sarcoplasmic K_{ATP} channels while openning of the mitochondrial K_{ATP} channels in neural stimulation which occures in epilepsy. With the help of the results obtained, we are targetting to determine the role of mitochondrial channels. With this study, for the first time, it has been targetted to search for the role of bepridil, applied in three different doses, in epilepsy prior to and during seizure.

3. MATERIAL AND METHODS

3.1. Animal

In this study, wistar albino rats, 4-6 months old and 300-350 grams in weight, have been obtained from BAİBÜ (Experimental Animal Application and Research Center). Total of 51 animals have been used in this study.

The animals were kept in 22-25C° room temperature and %60-70 humidity rate on 12-hour light/dark period. Water and nutrition were fed ad libitum. The study has been conducted with permission obtained from the Ethics Board for Animal Studies of BAİBÜ (ethical committee no: 2012/19). Randomly selected animals have been divided into 7 groups (Table 1). Rats have been anesthetized with 1.2g/kg intraperitoneal (i.p.) urethane (supplied by Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA). Epileptic focus has been generated by means of intracortical (IC) application of penicillin at a dose of 500 IU/2 µl (I.E. Ulugay, Istanbul, Turkey). No drug has been applied on the control group. Medications have been applied on the before seizure groups 10 minutes prior to the penicillin application whereas they have been applied on seizure experiencing groups 10 minutes after to the penicillin application. All of the drug have been prepared on a daily basis and intravenously injected into the tail vein. Bepridil has been disolved in ethyl alcohol and then applied on both before seizure and seizure experiencing groups in doses of 0.1mg, 1mg, 10mg/kg/100µl.

Table 1. Experimental groups

Groups	Drugs	Dose	The location of Drug Administration	The time of Drug Administration	n
Control	Penisilin G	500 IU/2 µl	İ.C		8
BS* Bepridil 0.1 mg/kg	Bepridil	0.1mg/kg/100µl	İ.V	10 Min Before Penicillin	7
BS* Bepridil 1 mg/kg	Bepridil	1mg/kg/100 µl	İ.V	10 Min Before Penicillin	6
BS* Bepridil 10 mg/kg	Bepridil	10mg/kg/100 µl	İ.V	10 Min Before Penicillin	9
DS** Bepridil 0.1 mg/kg	Bepridil	0.1mg/kg/100 µl	İ.V	10 Min After Penicillin	7
DS** Bepridil 1 mg/kg	Bepridil	1mg/kg/100 µl	İ.V	10 Min After Penicillin	6
DS** Bepridil 10 mg/kg	Bepridil	3mg/kg/100 µl	İ.V	10 Min After Penicillin	8

*BS: Before Seizure **DS: During Seizure

3.2. Surgical Operation

Following the application of anesthesia using 1.2gr/kg intraperitoneal (i.p.) injection of urethane (supplied by Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA), all of the rats have been placed on stereotaxic device. Skull bone from the 2mm posterior and 3 mm lateral of left cerebral cortex bregma has been removed followed by the removal of dura mater. Using Hamilton microinjector (701N, Hamilton Co., Reno, NV, USA), 500IU/2 µl of penicillin G has been injected at a depth of 1.2 mm in order to constitute epileptic focus.

3.3. Electrophysiological Recording

ECoG recording has been collected (Chart 5.1.1 (AD Instruments, Australia) Australia) after placing of two Ag- AgCl ball electrodes on somatomotor cortex, lateral to the bregma suture on the left hemisphere. Following the collection of a 180-minute EcoG recording from each animal, analysis for these recordings have been performed via Chart 5.1.1 (AD Instruments, Australia) software tool (AD Instruments Pty Ltd, Castle Hill, NSW, Australia).

3.4. Statistical Analysis

Number of spike waves, average amplitudes, and the seizure onset times in the recordings, analyzed in five-minute periods, have been evaluated using the One-Way ANOVA statistical software tool and distinctions in between the groups have been determined accordingly. LSD has been used for the Post-Hoc test and $p<0.05$ values have been considered statistically significant.

4. RESULTS

Once the seizure onset times were evaluated, a distinction has been found in the before seizure (BS) groups. With respect to all other groups, epileptic seizure has started later in the group that 1mg/kg bepridil was applied prior to seizure ($p<0.05$). Significant amount of delays on the seizure onset times have been observed on the group that 1mg/kg of bepridil was applied prior to seizure with respect to the groups that 1 mg/kg and 10mg/kg of bepridil were applied during seizure (DS)($p<0.05$) (Figure 1).

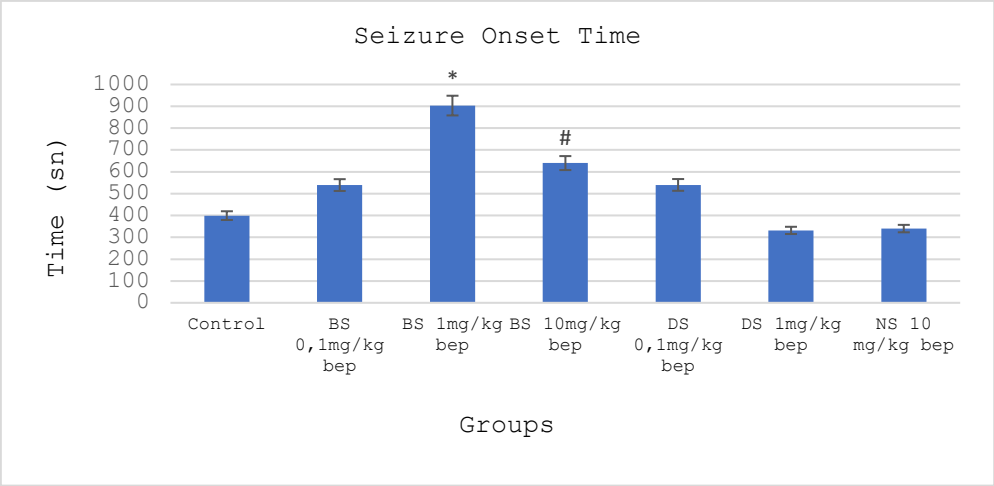

Figure 1. Seizure onset times (mean±SD)* is significantly different with respect to the control group# is significantly different with respect to the DS 10 mg/kg group ($p<0.05$)

1mg/kg BS group is statistically significant than other groups. 10mg/kg BS group is different than all DS groups from statistical significance point of view. Once the total number of spike waves is evaluated from groups' stand point for the duration of 180-minute recording period, a significant decrease in the number of spike waves has been observed in BS 10 mg/kg bepridil, DS 0.1mg/kg bepridil, and DS 1mg/kg bepridil applied groups with respect to the control group ($p<0.05$). Most effective dose of bepridil has been determined to be the 1mg/kg dose applied DS ($p<0.05$). Once examined in general, 10mg/kg dose applied DS has appeared to be ineffective ($p>0.05$) (Figure 2).

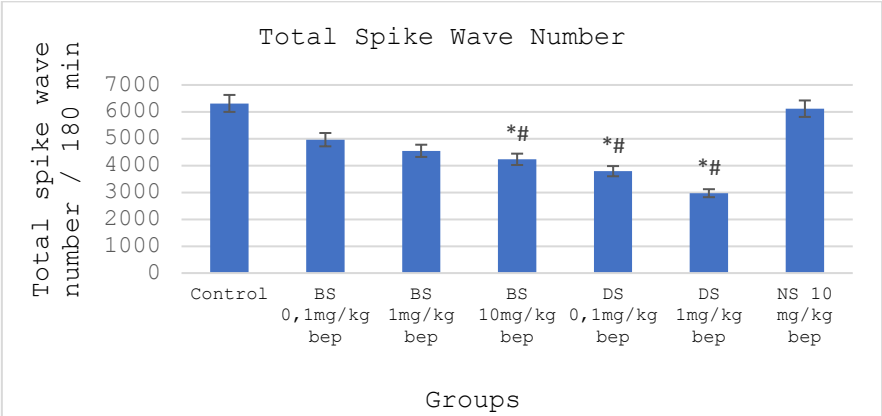

Figure 2. Total number of spike waves (mean±SD) *Total number of spike waves is significantly different with respect to the control group, #as well as they are significantly different with respect to the during seizure 10mg/kg group ($p<0.05$)

Once the total number of spike waves was evaluated for the initial 10 periods of the 180-minute recording duration, it has been observed that the seizures had started exactly after the 4th period. While the average number of spike waves was observed between 0 and 18 from the 4th through the 10th five-minute periods in the before BS 0.1mg/kg and before BS 10mg/kg bepridil-applied groups, the average is between 0 and 200 in DS 0.1mg/kg and DS 1mg/kg bepridil-applied groups. An increase of up to 100(0-100) in the number of spike waves has been observed in the before seizure (BS) 1mg/kg bepridil-applied

group, whereas in the during seizure (DS), the spike waves, has depicted a lower rate of increase after they started. In the BS 0.1mg/kg group; While decreasing the average number of spike waves between P13 and P17, it increased again between P18 and P21 ($p<0.05$). When the number of spike wave is high between the P22 and P25, spike wave number decreased significantly between P25 and P36 ($p<0.05$). Between P30 and P33, it decreased more and the seizure ended completely after P33 ($p<0.05$).

In the BS 1mg/kg group; While the average number of spike waves until the 10th period was close to the control group, It gradually decreased after P12 and it was observed that the number of spike waves between P14 and P20 decreased significantly ($p<0.05$). From P 21, it increased to the control level again, and the average spike wave number between P25 and P36 decreased significantly ($p<0.05$). Especially after P34, the seizure ended ($p<0.05$). From P 21, it increased to the control level again, and the average spike wave number between P25 and P36 decreased significantly ($p<0.05$). Especially after P34, the seizure ended ($p<0.05$). In the BS 10mg/kg group; Between P10 and P21, the mean spike wave number significantly decreased ($p<0.05$), and between P22 and P36, although it did not decrease to zero, it decreased compared to the control ($p<0.05$). In the DS 0.1mg/kg group; Spike Wave Number was significantly decreased between P10 and P21 ($p<0.05$). While the number of spike waves decreased significantly between P22 and P25 compared to the control, the Spike Wave Number between P25 and P36 remained constant even though it did not decrease to zero ($p<0.05$).

In the NS 1mg/kg group, bepridil significantly decreased Spike Wave Number between P10 and P21 ($p<0.05$). In this group, spike wave number between P22 and P25 was significantly lower than the control group, but it decreased further after P26 and stopped the seizure at P30 ($p<0.05$). In the NS 10mg/kg group, spike wave number did not differ from the beginning of the seizure to the end of the seizure compared to the control group ($p>0.05$) (Figure 3).

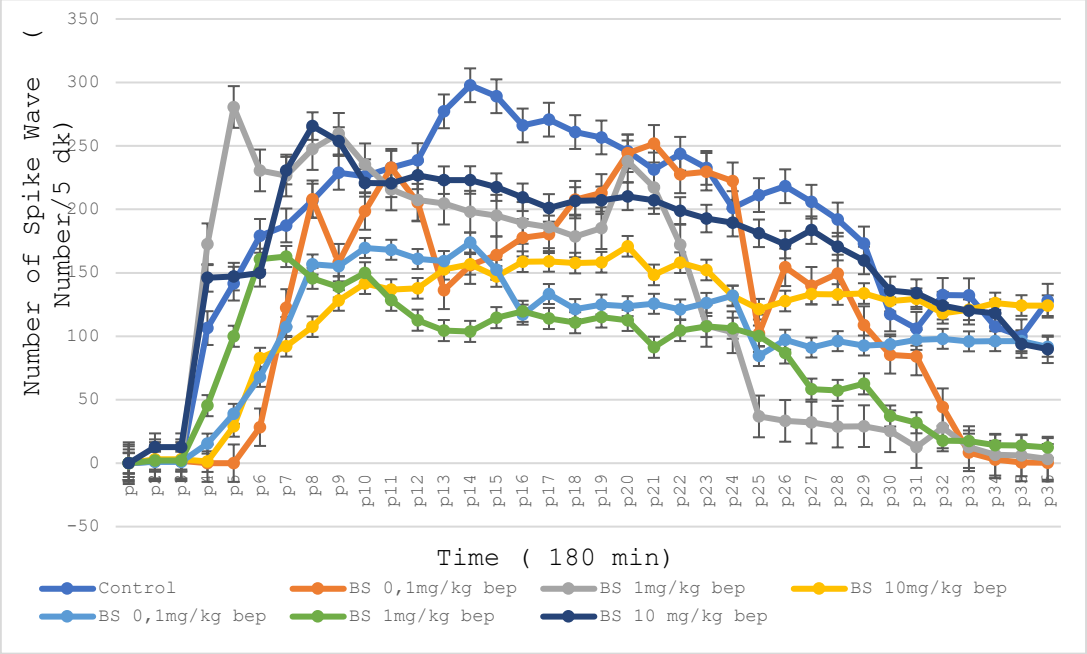

Figure 3. The average number of spike wave (mean±SD) for 5 minutes intervals in a total of 180 minutes of ECoG recording

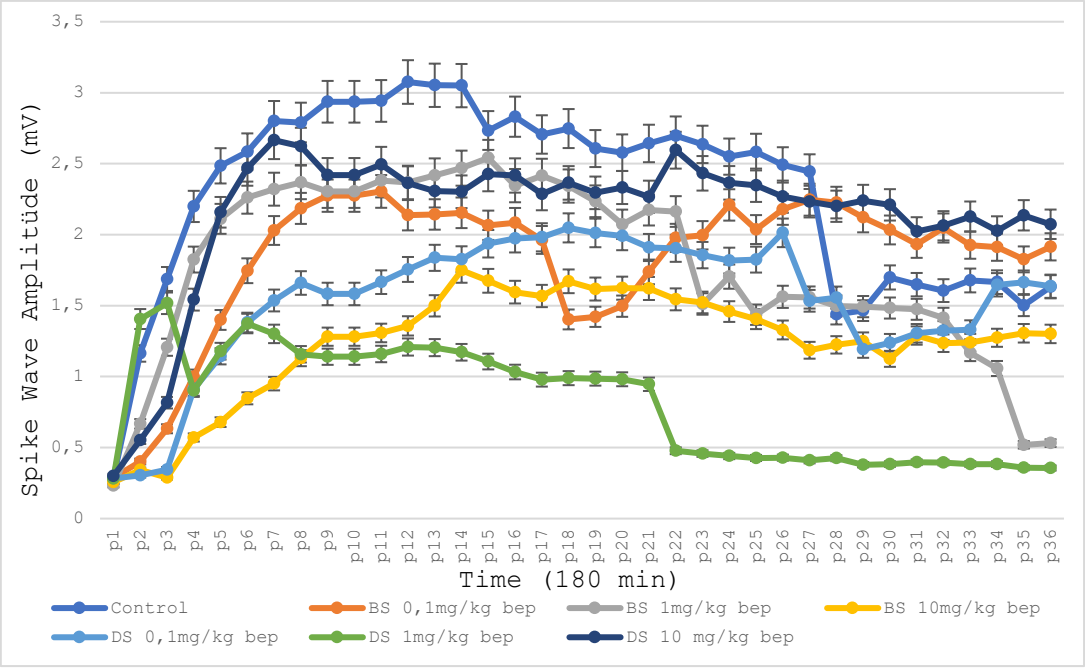

Figure 4. The average spike wave amplitude value (mean±SD) for 5 minutes intervals in a total of 180 minutes of ECoG recording

In the BS 0.1mg/kg group; The average amplitude of the spike waves between P1 and P10 was found to be significantly lower than the control group ($p<0.05$). Between P6 and P36, the amplitudes did not differ significantly compared to the control group ($p>0.05$).

In the BS 1mg/kg group; The average amplitude of spike waves between P1 and P36 is not significantly different from the control group ($p>0.05$).

In the BS 10mg/kg group; The average amplitude of the spike wave between P1 and P12 was found to be significantly lower than the control group ($p<0.05$).

In the DS 0.1mg/kg group; While the average spike wave amplitude values between P1-P14 are significantly lower than the control group, but there was no significant difference between P15-P36 and the control group ($p>0.05$).

In the DS 1mg/kg group; The average spike wave amplitude values between P1 and P36 are significantly lower than the control group ($p<0.05$).

In the DS 10 mg/kg; No significant difference was found between P1 and P36 in terms of average spike wave amplitude compared to the control group ($p>0.05$) (Figure 4).

5. DISCUSSION

Bepridil blocks the channels on the cell membrane called sarcolemmal K_{ATP} channels, while opening the mitochondrial K_{ATP} channels [13]. So far, there were no studies on the effect of bepridil on epilepsy. In this study, three different doses of bepridil were administered both before and during a seizure to investigate its effect on epilepsy, and it was found to decrease spike-and-wave frequency and stopped the seizures in a short time at doses of 0.1 and 1mg/kg. In the before seizure groups, it delayed the onset of seizures at doses of 0.1 and 1mg/kg. Neuronal K_{ATP} channels control the energy level in general, and neuronal ATP consumption adjusts the metabolic state of neurons precisely in line with the electrical activity. The ATP/ADP ratio depends on the increased regional activity, and changes

the channel activity. Activity-dependent stimulation of K_{ATP} channels hyperpolarizes the cells and reduces the excitability of neurons. K_{ATP} channel activity is associated with energy consumption and electrical signals, and is controlled by a negative feedback mechanism [14]. In this study, the reduction of spike-and-waves may have occurred due to the effect of K_{ATP} channels on the electrical activity. According to the literature, while sarcoplasmic K_{ATP} channels are associated with electrical activity, mitochondrial channels may be protective against cell death [15, 16 and 17]. The onset and progression of hypoxic/ischemic neuronal death is caused by excisotoxicity due to excessive glutamate release [18].

Activation of K_{ATP} channels during acute ischemic period can decrease glutamate release by reducing depolarization. In addition, somatodentritic K_{ATP} channel activation may reduce the onset of necrotic and apoptotic cell death in cases where intracellular Ca^{+2} increases extremely due to glutamate [19]. Some studies have found that K_{ATP} channel activation reduces neurotransmitter release. In addition, its partial effect on GABAergic neurotransmission has also been proven [18]. Animal studies have shown that bepridil has an antiarrhythmic activity. Bepridil shows a protective effect in myocardial cells by prolonging the duration of action potential [13 and 19]. Similar to this mechanism, this study shows that bepridil delays the onset of a seizure, when administered before a seizure, possibly by extending the duration of action potential, through a mechanism similar to that of myocardial cells. While bepridil protects the brain against ischemic damage by blocking K^+ outflow by blocking the sarcoplasmic channels, it also shows a protective effect at the cellular level by simultaneously opening mito K_{ATP} channels [13]. In this study, the seizure stopped in a short time compared to the control groups, when bepridil administered both before and during a seizure.

During a seizure, amount of intracellular ATP decreases due to a convulsant agent, hyperoxia, and hypoxia, and causes cell death due to activation of many pathways, such as increased amount of intracellular Ca, glutamate exitotoxicity, and inflammation. While cell loss increases the excitability of the cells here, it reduces the threshold and causes seizures to occur more easily. At this stage, K_{ATP} channels regulate the electrical activity of the cell by opening the sarcolemmal channels in relation with the decrease in the amount of intracellular ATP, and the cell hyperpolarizes with the loss of K in the cell in the first stage. On the other hand, the opening of the mitochondrial channels reduces cell death and exhibits a protective effect at the cellular level [16, 17 and 20]. The mito K_{ATP} channels are located in the inner mitochondrial membrane, control the mito volume, and play a role in protecting the cell [21]. Both K_{ATP} channels are normally closed. In cases such as hypoglycemia, ischemia, hypoxia, these channels are opened [22 and 23]. Transport of K^+ in the cytoplasm to the mitochondria leads to Ca^{+2} channel blockade [24]. In clinical trials, bepridil has been used as a Ca^{+2} channel blocker [13 and 21]. Bepridil, which causes potassium to be removed from the cytoplasm, also blocks the Ca^{+2} channels parallel to this effect, which explains its anti-convulsant effect. As shown in many studies of the Pentylene Tetrazol (PTZ) and Kainic acid induced epilepsy models, the K_{ATP} channel blockers (e.g. glibenclamide, 4-aminopyridine) trigger a seizure and shorten the latent period, while openers provide protection against a seizure and prolong the latent period [19, 23 and 25]. When administered before a seizure, bepridil increased the latent period, depending on the dose.

Intracellular ATP concentration decreases during excessive neuronal activation during seizures. It has been shown that agents that significantly decrease the amount of intracellular ATP have anti-convulsant effects through K_{ATP} channels. This mechanism shows an anti-convulsant effect by opening potassium channels when mitochondrial ATP levels drop. A study with cannabioids suggested that seizure threshold can be controlled by controlling mitochondrial K_{ATP} channels [26]. The mechanism of K^+ current regulation in the central nervous system can be performed by neurons [27, 28, 29 and 30]. A relationship has been shown between K^+ channels and epilepsy. In epilepsy, K channels directly control neural excitability while indirectly having effects on ion metabolism [30]. In-vivo and in-vitro pharmacological studies play a significant role in controlling seizures [31 and 32].

Depending on the change in extracellular ATP, many symptoms may occur, such as cerebral ischemia, stroke, and seizure [33 and 34]. In neuropathological cases in the brain, the metabolites resulting from extracellular ATP function have a complex effect, but they play a role in neuroinfilamtion, hyperexcitability and neurodegeneration [34, 35, 36 and 37]. In addition, extracellular change in ATP can cause neurological and neuropsychiatric diseases, including epilepsy [38 and 39]. Intracellular ATP concentration is of quite importance for the K_{ATP} channels. As a result of the decrease in the amount of intracellular ATP, these channels open and perform their function. Changes in extracellular ATP, the changes in neurons, and metabolic changes in the extracellular environment, or the changes due to any disease condition can affect the amount of intracellular ATP, and indirectly activate K_{ATP} channels.

6. CONCLUSION

In this study, it was observed that bepridil stops seizures in a short time at doses of 0.1 and 1mg/g when administered before and during a seizure. It was concluded that this drug may be used in the control of epilepsy since it delays the onset of seizures when administered before the seizures. In addition to this, it is important to note that bepridil acts by opening the mito K_{ATP} channels. If these effects are observable when mito K_{ATP} channels are opened, drugs that stimulate the opening of mitochondrial channels may be considered in new epilepsy treatment strategies. Although there were no data at the cellular level in this study, it is possible to assume that the mitochondrial K_{ATP} channels may also have a role in the electrical activity of the cell, according to the electrophysiological data, contrary to the literature. Previous studies have also proven the role of K_{ATP} channels in seizure control. Determining the specific role of mitochondrial K_{ATP} channels in electrical discharges during epilepsy is the original aspect of this study.

ETHICAL COMMITTEE APPROVAL

All experimental animals have been treated based on the guiding principles approved by the animal ethical committee of Bolu Abant Izzet Baysal University as well as all the treatments comply with recommendations provided on the Declaration of Helsinki (Registration number:2012/19).

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The effects of ATP –dependent potassium channel (KATP) agonist and antagonists on Penicillin G induced epilepsy model in rats.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Megiddo, I., Colson, A., Chisholm, D., Dua, T., Nandi, A., and Laxminarayan, R., (2016). Health and Economic Benefits of Public Financing of Epilepsy Treatment in India: An Agent-Based Simulation Model. Epilepsia, 57(3):464-474.

[2] Ngugi, A.K., Bottomley, C., Kleinschmidt, I., Sander, J.W., and Newton, C.R., (2010). Estimation of the Burden of Active and Life-time Epilepsy: A Meta-analytic Approach. Epilepsia, 51(5):883-890.

[3] Lasoń, W., Chlebicka, M., and Rejdak, K., (2013). Research Advances in Basic Mechanisms of Seizures and Antiepileptic Drug Action. Pharmacol Rep, 65(4):787-801.

[4] Dhopeshwarkar, A. and Mackie, K., (2014). CB2 Cannabinoid Receptors as a Therapeutic Target-what does the Future Hold?. Mol Pharmacol, 86(4):430-437.

[5] Noma, A., (1983). ATP-regulated K+ Channels in Cardiac Muscle. Nature, 305(5930):147-148.

[6] Ashcroft, F.M., Harrison, D.E., and Ashcroft, S.J., (1984). Glucose Induces Closure of Single Potassium Channels in Isolated Rat Pancreatic Beta-Cells. Nature, 312(5993):446-448.

[7] Bennett, K., James, C., and Hussain, K., (2010). Pancreatic β-Cell KATP Channels: Hypoglycaemia and Hyperglycaemia. Rev Endocr Metab Disord, 11(3):157-163.

[8] Flanagan, S.E., Clauin, S., Bellanné-Chantelot, C., de Lonlay, P., Harries, L.W., Gloyn, A.L., et al., (2009). Update of Mutations in the Genes Encoding the Pancreatic Beta-cell K(ATP) channel Subunits Kir6.2 (KCNJ11) and Sulfonylurea Receptor 1 (ABCC8) in Diabetes Mellitus and Hyperinsulinism. Hum Mutat, 30(2):170-180.

[9] Mannhold, R., (2004). KATP channel openers: structure-activity relationships and therapeutic potential. Med Res Rev, 24(2):213-266. doi:10.1002/med.10060.

[10] Liss, B. and Roeper, J., (2001). A Role for Neuronal KATP Channels in Metabolic Control of the Seizure Gate. Trends in Pharmacological Science, 12-22.

[11] Ardehali, H. and O'Rourke, B., (2005). Mitochondrial KATP Channels in Cell Survival and Death. J Mol Cell Cardiol, 39:7-16.

[12] Olson, T.M. and Terzic, A., (2010). Human KATP Channelopathies: Diseases of Metabolic Homeostasis. Eur J Physiol, 460:295-306.

[13] Jazayeri, A., Zolfaghari, S., and Ostadhad, S., (2013). Anticonvulsant Effect of Diazoxide against Dichlorvos- Induced Seizures in Mice. The Scientific World Journal, 1-4.

[14] Heurteaux, C., Bertaina, V., Widmann, C., and Lazdunski, M., (1993). K+ Channel Openers Prevent Global Ischemia-induced Expression of c-fos, c-jun, Heat Shock Protein, and Amyloid Beta-Protein Precursor Genes and Neuronal Death in Rat Hippocampus. Proceeding of the National Academy of Science USA, 90:9431-9435.

[15] Jiang, K., Shui, Q., Xia, Z., and Yu, Z., (2003). Changes in the Gene and Protein Expression of KATP Channel Subunits in the Hippocampus of Rats Subjected to Picrotoxin-induced Kindling.

Progress in Biophysics & Molecular Biology, 81:133-176.
[16] Yamada, K. and Inagaki, N., (2005). Neuroprotection by KATP Channels. The Journal of Molecular and Cellular Cardiology, 38:945-949.
[17] Takanari, H., Honjo, H., Takemoto, Y., Suzuki, T., Kato, S., Harada, M., et al., (2011). Bepridil Facilitates Early Termination of Spiral-wave Reentry in Two-dimensional Cardiac Muscle through an Increase of Intercellular Electrical Coupling. Journal of Pharmacological Sciences, 115:15-26.
[18] Gonca, E. and Bozdoğan, O., (2010). Both Mitochondrial KATP Channel Opening and Sarcolemmal KATP Channel Blockage Confer Protection Against Ischemia/reperfusion Induced Arrhythmia in Anesthetized Male Rats. Journal of Cardiovascular Pharmacology and Therapeutics, 15:403-11.
[19] Wang, J., Li, Z., Feng, M., Ren, K., Shen, G., Zhao, C., et al., (2013). Opening of Astrocytic Mitochondrial ATP-Sensitive Potassium Channels Upregulates Electrical Coupling between Hippocampal Astrocytes in Rat. Brain Slices, 8:1-14.
[20] Nichols, C.G., (2006). KATP Channels as Molecular Sensors of Cellular Metabolism. Nature, 440:470-476.
[21] Xue, Y., Xie, N., Lin, Y., Xu, J., Han, Y., Wang, S., et al., (2011). Role of PI3K/Akt in Diazoxide Preconditioning against Rat Hippocampal Neuronal Death in Pilocarpine-induced Seizures Brain Research, 1383:135–140.
[22] Ghasemi, M., Shafaroodi, H., Karimollah, A.R., Gholipour, T., Nezami, B.G., Ebrahimi, F., et al., (2010). ATP-sensitive Potassium Channels Contribute to the Time-dependent Alteration in the Pentylenetetrazole-induced Seizure Threshold in Diabetic Mice. Seizure, 19:53–58.
[23] Haj-Mirzaian, A., Ramezanzadeh, K., Afshari, K., Mousapour, P., Abbasi, N., Haj-Mirzaian, A., et al., (2019). Activation of ATP-sensitive K-channel Promotes the Anticonvulsant Properties of Cannabinoid Receptor agonist through Mitochondrial ATP Level Reduction. Epilepsy & Behavior, 93:1–6.
[24] Terzic, A., Dzeja, P.P., and Holmuhamedov, E.L., (2000). Mitochondrial KATP Channels: Probing Molecular Identity and Pharmacology, J Mol Cell Cardiol, 32:1911-5.
[25] Jan, L.Y. and Jan, Y.N., (1994). Potassium Channels and Their Evolving Gates. Nature, 371:119–22.
[26] Mathie, A., Wooltorton, J.R., and Watkins, C.S., (1998). Voltage-activated Potassium Channels in Mammalian Neurons and their Block by Novel Pharmacological Agents. Gen Pharmacol, 30:13–24.
[27] Storm, J.F., (1990). Potassium Currents in Hippocampal Pyramidal Cells. Prog Brain Res. 83:161–87.
[28] Köhling, R. and Wolfart, J., (2016). Potassium Channels in Epilepsy. Cold Spring Harb Perspect Med, 6:a022871.
[29] Niaki, S.E.A., Shafaroodi, H., Ghasemi, M., Shakiba, B., Fakhimi, A., and Dehpour, A.R., (2008). Mouth Breathing Increases the Pentylenetetrazole-induced Seizure Threshold in Mice: a Role for ATP-sensitive Potassium Channels. Epilepsy Behav, 13:284–9.
[30] Shafaroodi, H., Asadi, S., Sadeghipour, H., Ghasemi, M., Ebrahimi, F., Tavakoli, S., et al., (2007). Role of ATP-Sensitive Potassium Channels in the Biphasic Effects of Morphine on Pentylenetetrazole-induced Seizure Threshold in Mice. Epilepsy Res, 75:63–9.
[31] Franke, H., Grummich, B., Härtig, W., Grosche, J., Regenthal, R., Edwards, R.H., et al., (2006). Changes in Purinergic

Signaling after Cerebral Injury – involvement of Glutamatergic Mechanisms? Int. J. Dev Neurosci, 24:123–132.
[32] Melani, A., Turchi, D., Vannucchi, M.G., Cipriani, S., Gianfriddo, M., and Pedata, F., (2005). ATP Extracellular Concentrations are Increased in the Rat Striatum during in Vivo Ischemia. Neurochem Int, 47:442–448.
[33] Burnstock, G., (2016). An Introduction to the Roles of Purinergic Signalling in Neurodegeneration, Neuroprotection and Neuroregeneration. Neuropharmacology, 104:4–17.
[34] Enge, T., Gomez-Villafuertes, R., Tanaka, K., Mesuret, G., Sanz-Rodriguez, A., Garcia-Huerta, P., et al., (2012a). Seizure Suppression and Neuroprotection by Targeting the Purinergic P2x7 Receptor During Status Epilepticus in mice. FASEB J, 26:1616–1628.
[35] Jimenez-Pacheco, A., Diaz-Hernandez, M., Arribas-Blazquez, M., Sanz-Rodriguez, A., Olivos-Oré, LA., Artalejo, AR., et al., (2016). Transient P2x7 Receptor Antagonism Produces Lasting Reductions in Spontaneous Seizures and Gliosis in Experimental Temporal Lobe Epilepsy. J. Neurosci, 36:5920–5932.
[36] Doná, F., Conceição, I.M., Ulrich, H., Ribeiro, E.B., Freitas, T.A., Nencioni, A.L.A., et al., (2016). Variations of Atp and İts Metabolites in the Hippocampus Of Rats Subjected to Pilocarpine-induced Temporal Lobe Epilepsy. Purinergic Signal, 12:295–302.
[37] Engel, T., Alves, M., Sheedy, C., and Henshall, D.C., (2016). ATP Ergic Signalling During Seizures and Epilepsy. Neuropharmacology, 104:140–153.
[38] Engel, T., Alves, M., Sheedy, C., and Henshall., D.C., (2012a). Seizure Suppression and Neuroprotection by Targeting the Purinergic P2x7 Receptor During Status Epilepticus in Mice. FASEB J, 26:1616–1628.
[39] Beamer, E., Gölöncsér, F., Horváth, G., Bekő, K., Otrokocsi, L., Koványi, B., et al., (2016). Purinergic Mechanisms in Neuroinflammation: an Update from Molecules to Behavior. Neuropharmacology, 104:94–104.