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Hayriye Soytürk
Bolu Abant Izzet Baysal University, hayriyesoyturk1@gmail.com, Bolu-Turkey
Şerif Demir
Düzce University, serifdemir19@hotmail.com, Düzce-Turkey
Ömer Bozdoğan
Bolu Abant Izzet Baysal University, bozdogan_o@ibu.edu.tr, Bolu-Turkey

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ORCID ID	0000-0002-0000-3768	0000-0002-2548-1969	0000-0001-5073-0691
Corresponding Author	Hayriye Soytürk		

INVESTIGATION OF THE THERAPEUTIC AND PROTECTIVE EFFECTS OF ATP SENSITIVE POTASSIUM CHANNEL (K_{ATP}) AGONISTS AND ANTAGONISTS ON PENICILLIN INDUCED EXPERIMENTAL EPILEPSY MODELS IN RATS

ABSTRACT

Epilepsy is a disease occurring because of extreme activation of nervous system. Increased glutamate and Ca^{++} in brain is another reason of epilepsy. In this study, we investigated the effects of ATP sensitive potassium channel (K_{ATP}) agonists and antagonists on penicillin induced epileptiform activity in male wistar albino rats. Rats were divided three experimental main groups; (1) Control, (2) Before seizures (BS) groups, (3) During seizure (DS) groups. DS and BS groups are divided into four subgroups; (a)5HD, (b)HMR1098, (c)Bepridil (d)P 1075. Bepridil and p1075, reduce the number of spike-waves, while the effect of Bepridil appears to have a similar effect when administered both during and before the seizure. HMR1098 and 5-HD both increased the seizures both when administered before or during the seizure, different from the other studies. When K ATP channel agonists are administered before and during the seizure, they reduce the seizures, while antagonists increase the seizure.

Keywords: Epilepsy, mito K_{ATP}, sarc K_{ATP}, 5-HD, HMR 1098

1. INTRODUCTION

Epilepsy is one of the neurological diseases that is commonly seen in the world [1]. It is characterized by excessive activation of neurons that can not be controlled by the central nervous system. The balance between excitation and inhibition shifts to the excitation side during epileptic seizure. It has been proposed that, calcium ions play an important role in the formation of epilepsy. Calcium (Ca^{++}) entry into the cell leads to the release of excitatory neurotransmitters [2 and 3]. ATP sensitive potassium (K_{ATP}) channel modulation is related with the epilepsy. K_{ATP} channels have been found first in the heart [4]. They are widely distributed to many types of tissue and cell types. While K_{ATP} channels are inhibited by intracellular ATP, they are activated by ADP [5 and 6]. Interior region of the cells are sensitive to changes in adenine nucleotide concentration. K_{ATP} channels is thought to have an important role in various condition, such as hyperglycemia, hypoglycemia, ischemi, reperfusion [7] Different characteristics of K_{ATP} channel in many cell types has been identified in the brain comprise in hippocampal cells, in glia cells, in the dorsal vagal cell, in hypothalamic neurons and

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in the substantia nigra (SNR) [8]. Opening of K_{ATP} channels have been shown to be protective against neuronal damage and neurodegeneration during metabolic stress [9]. K_{ATP} channel activity is regulated by cellular metabolism [10 and 11]. K_{ATP} channels are found in pre and post synaptic neurons that exist in many regions of the brain. K_{ATP} channels can be opened or closed according to the changes in intracellular ATP/ADP ratio. When ATP level decrease, these channels open and K^+ leaks out of the cell and hyperpolarization takes place [12 and 13]. This condition is associated with the electrical activity of cells. Pharmacological studies have proven that K_{ATP} channels play an important role in controlling seizure threshold [5]. In experimental model of epilepsy induced with pentylenetetrazol (PTZ) and 4-aminopyridine (4a-AP: Aminopyridine) it was determined that K_{ATP} channel agonists (such as diazoxide, cromakalim, pinacidil, levcromakalim, nicorandil, minoxidil etc.) decreases seizure [14], while K_{ATP} ion channel antagonists (glibenclamide etc.) improve [12] epileptic seizures. In other words, these agents change neuronal excitability by modulating the K_{ATP} ion channels. Although, the underlying mechanisms of K_{ATP} ion channel agonist and antagonist on epileptic seizure have not been fully understood, there are few studies investigating the effects of the K_{ATP} ion channel agonist and antagonist in Penicillin induced epilepsy model [15 and 16]. K_{ATP} ion channel agonist cromakalim and antagonist 5-HD were determined to have different effects in diabetic rat with epilepsy model induced by PTZ [16].

2. RESEARCH SIGNIFICANCE

In this study, we aimed to investigate the effect of selective K_{ATP} channel agonists and antagonists on the initiation and severity of epileptic activity. This study will help us better understand the effect of ATP dependent mitochondrial and sarcoplasmic channel agonist and antagonists on the appearance and the duration of epileptic activity. The result of this study is also expected to provide some data to the clinical treatment of epilepsy in human beings.

3. MATERIAL AND METHODS

3.1. Animal

All experimental animals have been treated based on the guiding principles approved by the animal etihical comittee of Bolu Abant Izzet Baysal University as well as all the treatments comply with recommendations on the Declaration of Helsinki (Registration number 2012/19). Experiment was performed on 59 Male Wistar-Albino rats with weighing between 300-350 grams. Animals were housed under a 12L/12D light dark cycle (light on 07:00h) and room temperature of 22-25C°. All experiments were performed between 10:00 and 16:00h. They were given free access to food and water. Rats were divided to the following three experimental main groups; (1) Control, (2) Before seizures (BS)groups, (3) During seizure (DS)groups. DS and BS groups are divided into four subgroups; (a)5HD, (b)HMR1098, (c) Bepridil (d) P 1075. All animals were chosen randomly. 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. Drugs 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.

Table 1. Experimental Groups

Groups	Drugs	Dose	The location of Drug Administration	The time of Drug Administration	n
Kontrol	Penisilin G	500 IU/2µl	I.C		7
BS* 5HD	5HD	10mg/kg/100µl	I.V	10 Min Before Penicillin	7
BS* HMR1098	HMR1098	3mg/kg/100µl	I.V	10 Min Before Penicillin	7
BS* Bepridil	Bepridil	1mg/kg/100µl	I.V	10 Min Before Penicillin	6
BS* P1075	P 1075	0,1 mg/kg/100µl	I.V	10 Min Before Penicillin	7
DS** 5HD	5HD	10mg/kg/100µl	I.V	10 Min After Penicillin	7
DS** HMR 1098	HMR 1098	3mg/kg/100µl	I.V	10 Min After Penicillin	7
DS** Bepridil	Bepridil	1 g/kg/100µl	I.V	10 Min After Penicillin	6
DS** P1075	P1075	0,1g/kg/100µl	I.V	10 Min After Penicillin	5

*B.S:Before Seizure **D.S:During Seizure

3.2. Induction of Epileptiform Activity

All rats were anesthetized with 1.2 gr/kg urethane applied intraperitonealy and they were placed in stereotaxic frame prior to surgical operation. The left cerebral cortex was exposed to craniotomy (2mm posterior to bregma and 3mm lateral to saggitial). Then, dura mater was removed carefully after skull bone had been removed completely. The epileptic focus was produced by 500IU/2,5 µl penicillin G injection (1,2 mm beneath the brain surface by a hamilton micro syrınge).

3.3. Elecrtrophysiological Recordings

ECoG record was taken by two Ag/AgCl ball electrodes and earth connection was made by an Ag/AgCl clamp electrode for grounding. In this process of electrophysiological recording, first electrode;2mm lateral to sagittial suture and 1mm anterior to bregma; second electrode;2mm lateral to sagittial surutre and 5mm posterior to bregma and the grounding electrode was placed on left ear. Activity taken from electrodes was amplified by BioAmp (ADInstruments, Australia) interface and transferred to PowerLab 5/SP (ADI ınstruments, Australia) data recording system. PowerLab brain activity was viewed by Chart 5.1.1 (ADInstruments, Australia) software, and deposited in computer for post-experiment analysis.

3.4. Statistical Analyses

With the help of macro, which were included in Chart 5.1.1 (AD Instruments, Australia) program, the electrophysilogical recordings were analyzed following the transformation of electrophysilological recordings into numeric recording in 5minute intervals in terms of total spike waves number and mean amplitude in values. These analyses were conducted in 5minute intervals along the 180 minutes recording. Oneway ANOVA was used for statistical evaluations to compare the differences within groups. Significant results were tested by LSD post hoc test. Differences having p values lower than 0.05 were considered statistically significant. PASW 18.0 program was used for statistical calculations.

4. RESULTS

4.1. The Latency of Epileptic Activity Formation

Latency is the starting time of a seizure. Based on another definition, it is the time period between the administration of penicillin and the first spike wave. In the epilepsy studies, when investigating the therapeutic effect, drugs are applied administered after the onset of seizures, so the time of onset of seizures gives us information about whether the developed model has occurred. In the comparison of the onset of seizures for the DS groups in this study, there was no significant difference in the time of onset of seizures between the control group and other groups. In the BS groups, however, since the protective effect of drugs is investigated, the drugs are administered before the induced-epilepsy (before the penicillin administration), which can affect the time of onset of seizures. The fact that drugs delay the onset of seizures is one of the important evidence, showing that there may be a protective effect. In this study, the onset time of seizures was significantly higher in HMR1098 BS, Bepridil BS and P1075 BS groups in the BS groups than in the control group ($p<0.05$) (see Figure 1).

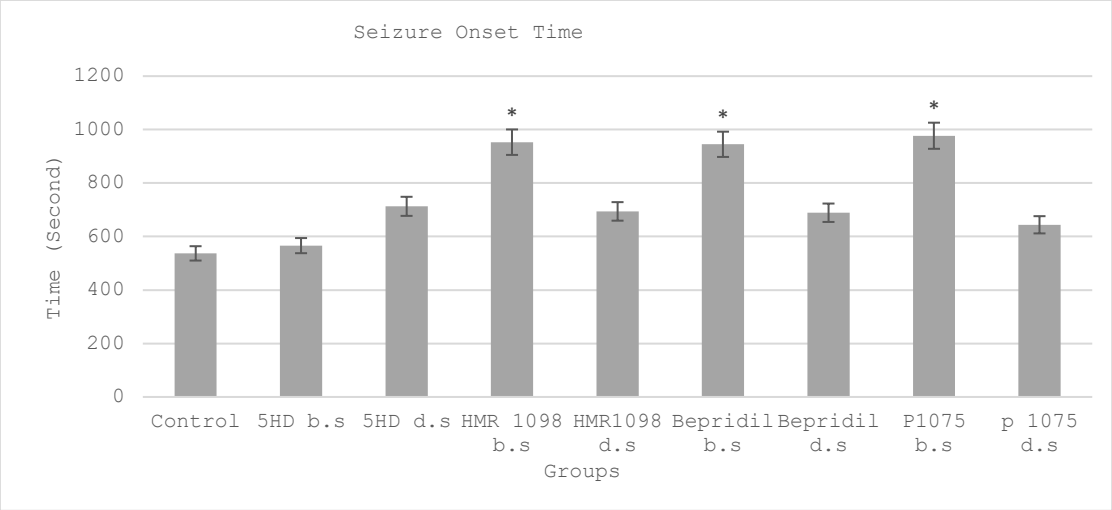

Figure 1. Seizure onset times (mean±SD)* is significantly different with respect to the control group ($p<0.05$)

4.2. Evaluation of the Number (Frequency) of Spike-waves

This study aims to investigate the therapeutic and protective role of K_{ATP} channels in penicillin-induced epilepsy. For this purpose, specific K_{ATP} agonists and antagonists were administered before and during the seizures. No differences were observed between basal activity records in all animals. A total of 180 min records were taken from each animal with the Power lab system, and 180 min was divided into 36-time intervals of 5 minutes. Each 5-minute time interval was called a period. Periods are denoted by "P". There was a significant difference between latent and spike wave numbers (frequency) when all groups were compared with the control group. The values of the experimental groups were expressed as mean±standard error (mean±SE) and the $p<0.05$ was considered statistical significance. When the number of spike wave numbers in 180 min records taken from a total of 9 groups, including Control, BS and DS groups and sub-groups, the total number of spikewave numbers in the HMR1098 DS Group was found to be significantly higher than the control group and Bepridil DS group. While Bepridil administration during the seizure significantly reduces

the number of spike waves, the administration of HMR1098 during the seizures significantly increased the number of spike waves ($p<0.05$). The 180 min records were divided into 36 periods of 5 minutes, and comparisons were made by evaluating the number of spike waves in each period. The BS and DS groups were compared separately with the control group. Before the seizure groups provided information about the protective effects of agonists and antagonists of K_{ATP} channels on epilepsy, while during the seizure groups provided information about its therapeutic effect.

4.3. Protective Effect of K_{ATP} Channel Agonist and Antagonist (BS)

In the comparison of Before the seizure (BS) groups with the control group, no significant differences were found between the groups until the 4^{th} period ($p>0.05$) (Figure 3). There was no significant difference between the number of spike waves in the control group and the number of spike waves in all other groups until the 25^{th} period ($p<0.05$) (Figure 2). While the number of spike waves in the 5HD BS group was lower than in the Bepridil BS group in the 5^{th} period ($p<0.05$), there was no significant difference with the other groups ($p>0.05$) (Figure 2). While the number of spike-waves in the HMR1098 BS group was significantly lower than in the Bepridil BS group in the 5^{th}-15^{th} period ($p<0.05$), there was no significant difference with the other groups ($p>0.05$). Between periods of 15-25, the number of spike waves in the HMR 1098 BS group was significantly higher than in the Bepridil BS and control groups ($p<0.05$) (Figure 2). In the Bepridil BS group, the number of spike waves between the 5^{th} and 11^{th} periods increased significantly compared to all other groups ($p<0.05$). While the number of spike waves between the periods 11 and 25 was equal to the control group ($p<0.05$), the number of spike waves between periods 25 and 36 was found to be decreased significantly in the Bepridil BS group compared to all other groups ($p<0.05$), (Figure 2). While the number of spike waves in P1075 BS group between the periods 5 and 11 was significantly lower than the Bepridil BS group, the number of spike waves in the P1075 BS group between periods 11-36 was significantly higher than the control and Bepridil BS groups ($p<0.05$) (Figure 2). P1075 is a mitochondrial channel opener, 5-HD is a mitochondrial channel blocker, and HMR1098 is a sarcoplasmic channel blocker. Bepridil, on the other hand, has a dual effect as both a sarcoplasmic channel blocker and a mitochondrial channel opener. The effects of P1075 BS, 5-HD BS and HMR1098 BS are similar when administered before the seizure.

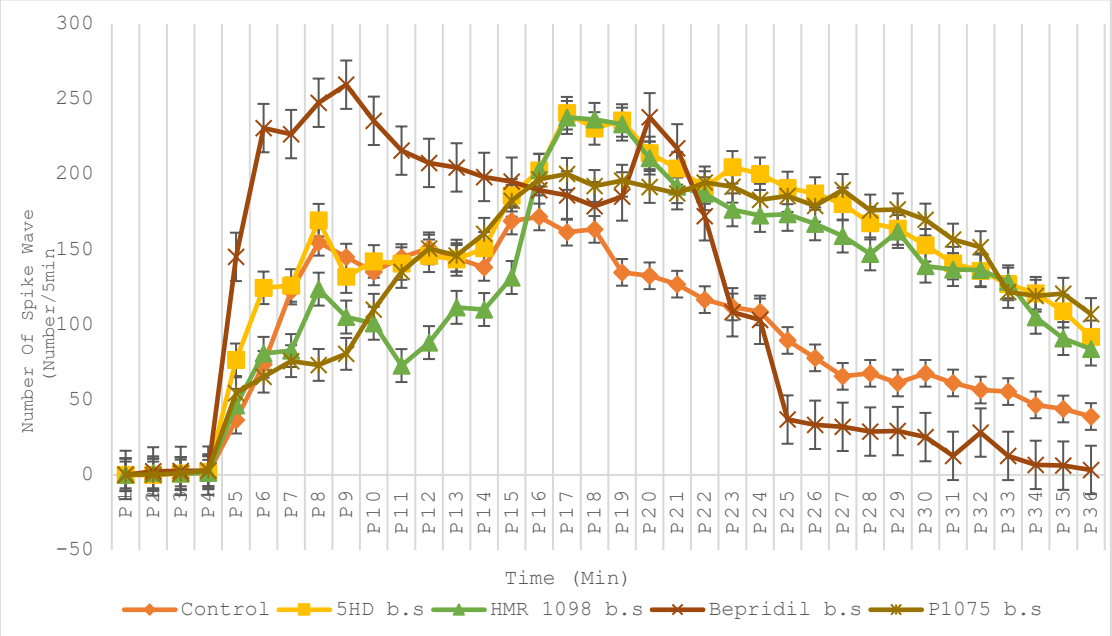

Figure 2. The average number of spike wave in before seizure groups (BS) (mean±SD) for 5 minutes intervals in a total of 180 minutes of ECoG recording

4.4. Therapeutic Effect of K_{ATP} Channel Opener and Blocker (DS)

There were no significant differences between the groups until the 25th period. The number of spike waves in the HMR1098 DS group was statistically higher than the Control, 5HD DS, Bepridil DS, and P1075 DS groups only in the 5th period ($p<0.05$) (Figure 3). The effects of all groups on seizures were similar. Until the 11th period, Bepridil increased the seizures, while after the 25th period, the effect reversed, acting more than all other groups, reducing the seizures more than all groups ($p<0.05$) (Figure 3). Bepridil BS has reduced the number of spike waves compared to the 5-HD BS, P1075 BS, and HMR1098 BS groups ($p<0.05$) (Figure 3). The effect of Bepridil DS appeared at the 27th period. Looking at the results, the Bepridil DS group significantly reduced the number of spike waves compared to the 5-HD DS and HMR1098 DS groups ($p<0.05$) (Figure 3). From the 28th period, the effect of the P1075 DS began to be seen, and again it was more effective in this group than the 5-HD DS and HMR1098 DS groups ($p<0.05$) (Figure 3).

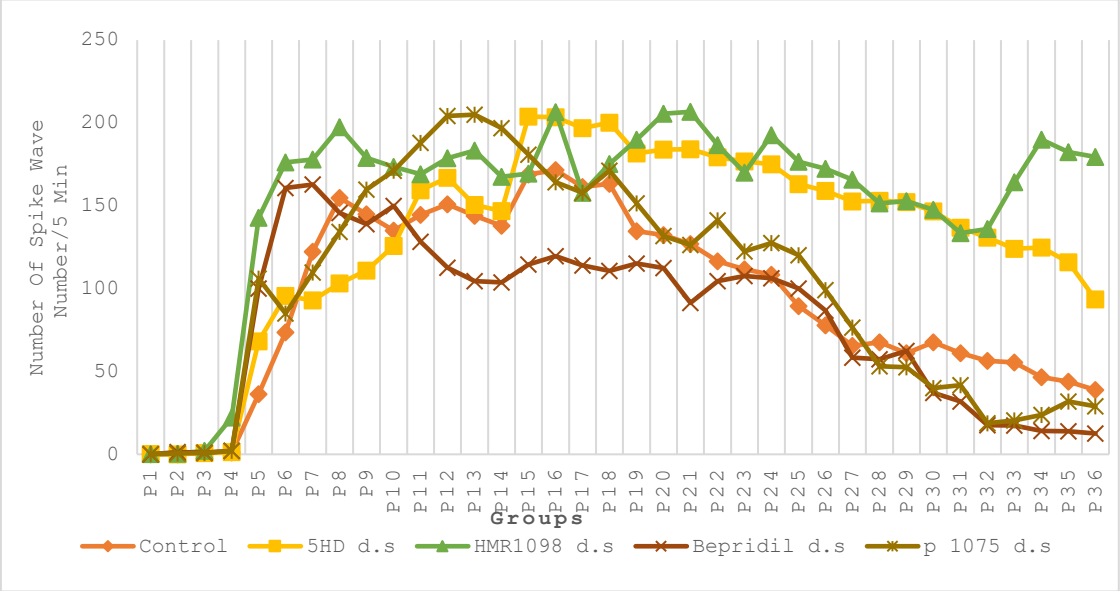

Figure 3. The average number of spike wave in during seizure groups (DS) (mean±SD) for 5 minutes intervals in a total of 180 minutes of ECoG recording

5. DISCUSSION

Epilepsy is a disease occurring because of extreme activation of nervous system. Increased glutamate and Ca^{++} in brain is another reason of epilepsy and it has been shown in many studies that it triggers neuronal cell death. It has been long confirmed by experimental models and clinical studies that a prolonged seizure or status epilepticus (SE) could cause neuronal death in the brain [17]. But, the detailed effect of K_{ATP} channel openers and blockers on neuronal death and apoptosis in cerebral ischemia reperfusion induced injury has not been fully understood, and whether mitoK_{ATP} channels play a more important protective role than sarcK_{ATP} channels in the brain is unclear. Epileptic seizures caused by ischemia and trauma lead to formation of excitotoxicity, free radicals, inflammation and cell apoptosis. Today antiepileptic drugs used in the treatment inhibits one or more of these destructive mechanisms which results in decrease or stop of cell damage and epilepsy. It is estimated that K_{ATP} openers used in this study, might decrease the cell damage by stopping one or more of these destructive mechanism [18].

In this study, ATP dependent potassium channel blockers increased the frequency of spike wave, otherwise opener decreased the frequency of spike wave during epileptic activity. In the literature, the effects of glybenclamide (K_{ATP} channel blocker/antagonists) and pinacidil (K_{ATP} channel opener/agonists) on the epileptic seizure were researched [16] Although not the identical drug were used, the result of this study had similarities to the previous studies in that Bepridil, an mito ATP dependent potassium channel agonists, decreased the duration and frequency of the epileptic activity, otherwise HMR1098, an ATP dependent K channel antagonists, increased the duration and frequency of epileptic activity.

Bepridil, which was used in this study, has a dual effect as a mitoK_{ATP} channel opener and a sarcK_{ATP} channel blocker. Since dose of Bepridil was not clear in the literature, it was studied in three different doses, and the results were compared to the control. Bepridil applied before epileptic seizure was effective on spike wave

frequency. But it was known that Bepridil closes sarcolemmal K_{ATP} channel and opens mitochondrial K_{ATP} channel [19]. Bepridil was found very effective to decrease arrhythmia induced by ischemia and reperfusion. In this study, Bepridil was found to be effective to decrease the spike wave frequency during epileptic seizure. The effect of Bepridil might be driven epileptic activity from its effect on the K_{ATP} channel in neurons. Since Bepridil is known to be effective on the ATP dependent potassium channel, possibly the spike wave occurring during epileptic seizure are related with the ATP dependent potassium channel modulation. It has been reported that Bepridil is an antianginal agent having vasodilator and direct cardiac effects, including negative chronotropic and inotropic effects [20]. Bepridil, in animal models, has robust anti arrhythmic activities. Studies showed that Bepridil has a protective effect and prolong the action potential duration in myocardial cell [21]. In the previous studies, it was shown that K_{ATP} channels couple with the intracellular metabolic state and this is important in the control of neuronal excitability and seizure propagation [22].

When administered before the seizure, Bepridil significantly increased the number of spike-waves compared to both the post seizure group of Bepridil and the antagonists. This effect of Bepridil may be due to its double effect. This is because, Bepridil has a double effect by opening mitochondrial channels while blocking sarcoplasmic channels. Bepridil may have increased depolarization by acting on the membrane potential by blocking the K_{ATP} channels as a sarcoplasmic channel antagonist until the 11^{th} period. In later periods, however, this dual effect may decrease the seizure falls below the control group and terminate at an early stage.

By opening only, the mitochondrial channels, Bepridil further reduced seizures compared to the control group. It similarly increased the spike wave activity of the mitochondrial channel opener or blocker applied before the seizure.

In CA1 decreases K^+ flux, depending on concentration in neurons [16] This result suggests that blockade of K currents by Bepridil contributes to the protection of brain against ischemic damage [23] Also, Bepridil provides its anti arithmic effect by opening mito K_{ATP} channels and closing sarcoplasmic channels [24]. Thus, K^+ in cytoplasm migrates to mitochondri and may lead to Ca^{++} channel blocakage [25]. Further studies should be need to understand the precise role of K inflow on the blockage of Ca^{++} channels. It was also observed by patch clamp techniques that Bepridil was effective to decrease K^+out flux [26].

SarcK_{ATP} channels that are found in the cell membrane, controls electrical activity of the brain [12]. MitoK_{ATP} channels that are found in inner mitochondrial membrane regulate the mitochondrial volume, as well as they have a role in protection of cell [12]. Both two types of K_{ATP} channels are normally in closed state. Under abnormal conditions, K_{ATP} channels in cells have tendency to open under metabolical stress (hypoclycemia, hypoxia, ischemia etc.).

Ischemic brain damage induced by carotid ligation is prevented by Bepridil. In these conditions, it was suggested that the membrane hyperpolarization is induced by hypoxia and anoxia, and extracellular K^+ concentration increases by activation of K^+ channels. Sarcolemmal blockage and mitochondrial opening of ATP dependent potassium channels are induced by Bepridil which showed to decrease brain injury [27]. Similar event might be effective in this study; Bepridil might decrease the electrical discharge induced by penicillin induced seizure and result in less frequency of spike during seizure.

P1075, a derivative of pinacidil, is a cytoplasmic K_{ATP} channel agonist. Up to date P1075 was used in ischemia/reperfusion studies done on heart [28].

In this study, it was found that HMR 1098 application during and before seizure increased spike wave frequency significantly. This means that HMR 1098 was also effective to the K_{ATP} channel in nerve cell. K_{ATP} channel antagonist, HMR 1098 has a prolongation effect on seizure. In addition, studies in heart showed that HMR 1098 shortened action potantial duration [29]. Plasma membrane sacolemmal K_{ATP} channels are blocked by this drug, but mitoK_{ATP} channels are not blocked [30]. Since HMR 1098 is a sarcoplasmic channel blocker, by closing K_{ATP} channels that are open during seizure, and it blocks K efflux and leads to Ca^{++} overload which means intracellular Ca^{++} amount increases and seizure gets more severe. When it is applied before the seizure, it decreases seizure threshold because of stimulation of Ca^{++} overload. In our study, HMR1098 increased the spike frequency when it is aplied to both during and before seizure which coincides with the electrophysiologic effect mentioned above.

When Sarcoplasmic K_{ATP} channels are closed, K^+ cannot leave out of the cell and Ca^{++} influx increases. Intracellular Ca^{++}in high levels activate degradative enzymes and cell goes into lysis. In this study, increase in intracellular Ca^{++} concentration makes the cell more positive (depolarization) which leads to easy stimulation of neurons. While increases in intracellular Ca^{++} concentration causes over excitation of neurons, it also damages the cell irreversibly by activating many degradative enzymes including apoptotic pathway [31].

5HD is a commonly used mitochondrial blocker and it selectively blocks mitoK_{ATP} channels. In intact cells, 5-HD fails to inhibit sarcK_{ATP} channels, suggesting that mitochondria are the targets of 5-Hydroxydecanoate (5-HD) that it inhibits the protective effect of ischemic preconditioning, and it is assumed to be a selective inhibitor of mitochondrial ATP sensitive K^+ (mitoK_{ATP}) channels [32].

In this study the effects of special drugs on penicillin induced epilepsy was researched. Results obtained in this study confirmed the results of the previous study so that K_{ATP} channel openers increased seizure threshold and decreased spike wave frequency [33]. In addition to the previous research, in this study it was investigated that the effects of Bepridil and P1075 on the seizure activity. According to these results, sarcoplasmic K_{ATP} channel openers had better positive effects on spike wave frequency, and amplitude and seizure initiation time than mitochondrial K_{ATP} channels openers. In addition, it has been found that selective mitochondrial blocker, and 5HD increased seizure activity. Selective opening of mitoK_{ATP} channels has neuroprotective effects against ischemia–reperfusion injury in the brain, neurons could be protected against death and injury by activation of mitoK_{ATP} channels [34].

6. CONCLUSION

When the result of this study is examined, it is seen that the effects of administrations before and during the seizures differ. Looking at all the groups, although Bepridil BS increased the number of spike-waves until 11th period, it reduced the seizures more than mito openers and blockers starting from the 25th period. Opening of sarcoplasmic channels (with Bepridil) in the Before-the-seizure groups significantly reduced seizure, while blocking of sarcoplasmic channels (with HMR1098) in the During-the-seizure groups increased the seizures much more than control and other groups, even in the 36th period. According to these results, we can state that sarcoplasmic K_{ATP}

channels are more effective in seizure control (Figure 2 and Figure 3). Looking at the administration of the drugs after the seizure, in other words, when we look at the therapeutic effect of the drugs, although Bepridil did not reduce the number of spike-waves much compared to the control group, it started to reduce the number of spike-waves after the 30th period compared to the control and other groups in both agonists. hmr1098 (sarcoplasmic blocker) and 5-HD (mitochondrial blocker), the specific antagonists, significantly increased the number of spike-waves compared to the control and agonists.

Bepridil appears to be effective in reducing the number of spike-waves in the Before-the-seizure groups, and both Bepridil and P1075 are effective in reducing the spike-waves in the During-the-seizure groups. Bepridil shows this effect by opening mitochondrial channels while closing the sarcoplasmic channels. P1075, however, is effective on mitochondrial channels. In conclusion, we clearly see that opening of the mitochondrial channels plays a role in the early termination of seizures. Similar to the literature, this study also showed that opening KATP channels reduces seizures, while closing K_{ATP} channels increases seizures.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

FINANCIAL DISCLOSURE

The authors declare that this study has received no financial support.

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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