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Activation of ATP-sensitive K-channel promotes the anticonvulsant properties of cannabinoid receptor agonist through mitochondrial ATP level reduction☆

Arvin Haj-Mirzaian [a,b,c,1], Kiana Ramezanzadeh [a,b,c,1], Khashayar Afshari [a,b], Pouria Mousapour [a,b,c], Nooshin Abbasi [a,b,d], Arya Haj-Mirzaian [a,b,e], Rajan Nikbakhsh [a,b,c], Nazgol-sadat Haddadi [a,b], Ahmad Reza Dehpour [a,b,*]

[a] Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
[b] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Interdisciplinary Neuroscience Research Program (INRP), Tehran University of Medical Sciences (TUMS), Tehran, Iran
[e] Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA

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ABSTRACT

Cannabinoid receptor (CBR) agonist could act as a protective agent against seizure susceptibility in animal models of epilepsy. Studies have shown that potassium channels could play a key role in ameliorating neuronal excitability. In this study, we attempted to evaluate how CBRs and Adenosine Tri-Phosphate (ATP)-sensitive potassium channels collaborate to affect seizure susceptibility by changing the clonic seizure threshold (CST). We used male Naval Medical Research Institute (NMRI) mice and treated them with the following drugs: cromakalim (a potassium channel opener, 10 μg/kg), glibenclamide (a potassium channel blocker, 0.03 and 1 mg/kg), 0.5 mg/kg of AM-251 (a selective CB1 antagonist), AM-630 (a selective CB2 antagonist), and 0.5, 3, and 10 mg/kg of WIN 55,212-2 (a nonselective agonist of CBRs); and CST was appraised after each type of administration. Also, we evaluated the ATP level of the hippocampus in each treatment to clarify the interaction between the cannabinoid system and potassium channel. Our results showed that administration of WIN 55,212-2 at 10 mg/kg significantly increased CST ($P < 0.001$). This change could be reversed by using AM-251($P < 0.001$) but not AM-630. Also, either cromakalim (10 μg/kg) or glibenclamide (0.03 and 1 mg/kg) could not significantly affect the CST. In addition, glibenclamide (1 mg/kg) could reverse the anticonvulsant effect of WIN 55,212-2 (10 mg/kg) on CST ($P < 0.001$). However, the anticonvulsant effect was observed when cromakalim (10 μg/kg) was added to WIN 55,212-2 at its subeffective dose (3 mg/kg) in comparison to single-treated animals. Interestingly, we observed that CB1 agonist could significantly decrease ATP level. In conclusion, CB1 agonist accomplishes at least a part of its anticonvulsant actions through ATP-sensitive potassium channels, probably by decreasing the mitochondrial ATP level to open the potassium channel to induce its anticonvulsant effect.

1. Introduction

Epilepsy is a chronic debilitating brain disorder, primarily characterized by recurrent seizures [1]. About 65 million people, particularly children and the elderly, are affected by epileptic seizures across the world. Considerably, epilepsy is involved in numerous diseases with different etiologies varying from brain trauma to genetic disorders. Moreover, plenty of patients with epilepsy are afflicted by various cognitive, psychological, and social problems, which undermine their quality of life [1,2].

Cannabinoid system including CB1 and CB2 receptors is indicated to be significantly involved in multiple normal or impaired Central Nervous System (CNS) functions including memory, cognition, appetite, analgesia, and motor tasks. In this regard, recent studies have suggested that CB1 receptors are the main receptor across cannabinoid receptors (CBRs) [3,4]. Past studies revealed that both endogenous and exogenous cannabinoids are capable of regulating various effects including seizure [5–9]. Numerous studies supported this notion that cannabinoids could

☆ Compliance with ethical standards: The authors declare that they have no conflict of interest. Also, all applicable international and institutional guidelines for the care and use of animals were followed.

* Corresponding author at: Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O.Box13145-784, Tehran, Iran.
E-mail address: dehpoura@sina.tums.ac.ir (A.R. Dehpour).

[1] The first two authors are considered as the first authors.

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protect against seizure, mostly through activation of CB1 receptor. In different models of animal epilepsy, such as maximal electroshock model of grand-mal seizure, the pilocarpine model of acquired epilepsy in rats, and the mouse pentylenetetrazole (PTZ) model of myoclonic seizures, a remarkable anticonvulsant effect was detected through CB1 receptor activation [7–10]. In addition to mentioned information, interestingly, past studies have shown that CBR agonist could inhibit the mitochondrial function [11]. Also, recent reports revealed that CB1 is functionally present at mitochondrial membranes (mtCB1) in the brain [12]. Thus, CBR agonist and antagonist could directly regulate intramitochondrial signaling and respiration through mtCB1 receptors. Notwithstanding, the underlying mechanisms of cannabinoids protective role against seizure remain to be investigated more extensively.

Currently, it has been revealed that CNS neurons are able to regulate potassium (K) currents through distinct modulatory mechanisms [13–15]. Evidence has demonstrated that K channels are correlated with epilepsy syndromes through different pathways including direct control of neuronal excitability and homeostasis of ion milieu to indirect effects via metabolism [16]. Potassium-channels have various types and subtypes such as calcium-activated K channel, voltage-gated K channel, ATP-sensitive K (K_{ATP}) channel, etc. [16]. ATP-sensitive K channels are composed of two major subunits including inwardly-rectifying K ($K_{ir}6.2$) and sulfonylurea receptor (SUR) and could present in various types of tissues such as the brain, muscle, pancreatic beta cells, etc. [16]. The activity of this channel regulates by intracellular level of ATP and adenosine diphosphate (ADP). It was well-recognized that high ATP level could close the K_{ATP}, and low level of ATP could open this channel [17]. There are several lines of evidence demonstrating that central CB1 receptor activation leads to decreased levels of cyclic adenosine monophosphate (cAMP) in neural cells via G-protein-dependent blockade of adenylyl cyclase [18–21]. Also, some evidence has shown the interaction between CBRs and K channels like K_{ATP} and inwardly rectifying potassium channel [22]. This evidence has demonstrated that cannabinoids could activate an $K_{ir}6.2$ current and also could alter K_{ATP} [22,23]. By taking together of this information, we hypothesized that CBR agonist as a mitochondrial inhibitor could alter the ATP level of the brain cells, and this condition might impact on the K_{ATP} channels and seizure threshold.

According to the abovementioned information, CBRs could interact with K_{ATP}-channel through mitochondrial ATP level of neuronal cells. Here in this study, we aimed to investigate the possible interaction of CBRs and K-channel through modulation of mitochondrial ATP level in their anticonvulsant effect in an animal model of seizure.

2. Material and methods

2.1. Animals and housing

In this study, we included 160 male Naval Medical Research Institute (NMRI) mice, weighed 20–26 g. Mice were housed in a room with controlled temperature (22 ± 1 °C), light (12-h regular light/dark cycle), and humidity (50 ± 10%). Animals had free access to standard laboratory, food, and water. Assignment of subjects to experimental groups (n = 10 in each group) was randomized. All experiments were done during the period between 9 a.m. and 1 p.m. The experimental procedures used throughout this study were in accordance with the guidelines for the animal care and use; and were approved by the Local Ethics Committee on Animal Experimentation of Tehran University of Medical Sciences.

2.2. Drugs

In this study, following drugs were used: cromakalim (10 μg/kg), as a K channel opener, dissolved in dimethyl sulfoxide (DMSO); and glibenclamide (GLI) (0.03 and 1 mg/kg), as a K channel blocker, dissolved in saline (0.9% NaCl). Glibenclamide (1 mg/kg) administrated intraperitoneally. As previously reported, these drugs do not significantly alter the plasma level of glucose. Both cromakalim and GLI were purchased from Sigma–Aldrich (Sigma, St. Louis, MO, USA). In addition, WIN 55,212-2 (a nonselective agonist of CBR), AM-251 (a selective CBR 1 antagonist), and AM-630 (a selective CB2 receptor antagonists) were purchased from Sigma–Aldrich (Sigma, St. Louis, MO, USA). AM-251 and AM-630 were dissolved in saline; WIN 55,212-2 was dissolved in DMSO and further diluted with 5% Tween-80 and 90% saline (Final DMSO concentration was 5%). Dimethyl sulfoxide, Tween-80, and saline solutions were used as vehicles (vehicle: 1:1:18 of DMSO, Tween-80, and saline, respectively). Injections were carried out through intraperitoneal (i.p.) route in a constant volume of 5 mL/kg body weight. 0.5 mg/kg of AM-251 was administrated 30 min before experiments; and WIN 55,212-2 (0.5, 3 and 10 mg/kg) was administered 60 min prior to the tests [24]. All doses and times of administrations of each drug were chosen based on pilot studies and previously published reports [24,25].

2.3. Seizure induction

Pentylenetetrazole was dissolved in heparinized sterile saline (0.9%) to prepare a fresh solution with a concentration of 5 mg/mL before an intravenous (IV) infusion with an infusion rate of 0.5 mL/min [26]. Before testing, each mouse was weighed, placed in a clear acrylic plastic restrainer, and its tail was immersed in a warm water bath (40–45 °C) for 1 min for the tail veins to dilate. A dental carpule (30 G) was connected to a 10-mL syringe prefilled with heparinized PTZ solution by a polyethylene tube (No. 10), and the needle was inserted into the midlength of the lateral tail vein. During the infusion, the animal was let free to move with the aid of attached cannula. Each animal was observed throughout the infusion process. The duration time between the start of infusion and onset of clonic seizures was recorded, converted to the clonic seizure threshold (CST) by the following formula [27].

$$CST = \frac{Concentarion\left(\frac{mg}{ml}\right) \times Infusion\ rate\left(\frac{ml}{min}\right) \times Time\ (s) \times 1000}{Weight \times 60}$$

2.4. Experimental design

At the first part of this study, we evaluated CST by using CBR agonist (WIN 55,212-2 at doses of 0.5, 3, and 10 mg/kg) and antagonists (AM-251 and AM-630 at doses of 0.5 mg/kg). On the other hand, we evaluated the role of each CBR by using coinjection of WIN 55,212-2 with AM-251 or AM-630. Additionally, the same experiments were done by administrating a K channel opener (cromakalim at 10 μg/kg) and blocker (GLI at doses of 0.03 and 1 mg/kg). In the final step, we try to investigate the relation between CBR and K channel on CST by using cotreatment of each drug which was mentioned above. In this manner, we coadministrated WIN 55,212-2 (10 mg/kg) with GLI (0.03 and 1 mg/kg), WIN 55,212-2 (10 mg/kg) with cromakalim (10 μg/kg), WIN 55,212-2 (10 mg/kg) with AM-251 (0.5 mg/kg), WIN 55,212-2 (10 mg/kg) with AM-251 (0.5 mg/kg) and GLI (1 mg/kg), and finally we coinjected WIN 55,212-2 (3 mg/kg) plus AM-251 (0.5 mg/kg) and cromakalim (10 μg/kg).

2.5. Assessment of mitochondrial ATP level

After scarifying animals in all our experimental groups, the hippocampus was dissected from the brain and then homogenized. Based on our previous publications, we homogenized the sample at 4 °C using cold mannitol solution containing 0.225-M D-mannitol, 75-mM sucrose, and 0.2-mM EDTA [28,29]. The homogenized samples were centrifuged at 1000 ×*g* for 10 min at 4 °C to remove the nuclei,

unbroken cells, and other nonsubcellular debris. Also, it should be noted that we did not use animals that were treated with PTZ for evaluating ATP level. Evaluation of ATP level was done after treating the animals with all effective treatments of this study.

After separating the supernatant, this part was centrifuged at 10,000 ×*g* for 10 min, as a source of prefrontal cortex (PFC) and hippocampus (HIPP) mitochondria. The heavy mitochondrial fraction was collected and resuspended in the mannitol solution, and recentrifuged twice at 10,000 ×*g* for 10 min. The resulting pellet (P2 fraction), including both synaptic and nonsynaptic mitochondria, was resuspended in the desired buffer based on mitochondrial function markers including ATP [30].

For analyzing the concentration of ATP, 0.5 mL of mitochondrial homogenate in TCA 6% were pooled with 0.5 mL of KOH 0.05 M, then, supplemented with 1 mL deionized water; after 2 min 650 μL of KH_2PO_4 (0.05 M) was added and vortexed. After filtering, ATP level in each sample was determined using luciferase enzyme based on criteria described previously [31]. Bioluminescence intensity was measured using Sirius tube luminometer (Berthold Detection System, Germany).

2.6. Statistical analysis

Comparisons between the groups were assessed using t-test and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using Graph Pad Prism 7 software (San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant. Also, we evaluated the power of each group analysis by using G*Power software version 3.

3. Results

3.1. Anticonvulsant role of CBRs

One-way ANOVA showed a significant alteration in CST of animals after treating the animals with CBR agents ($F(7, 72) = 2.957$, $P < 0.001$, Fig. 1). Postanalysis revealed that WIN 55,212-2 at lower doses (0.5 and 3 mg/kg) could not significantly increase CST ($P > 0.05$). Also, analysis failed to show any significant effects for other single treatments (AM-251 at 0.5 mg/kg and AM-630 at 0.5 mg/kg) and cotreatment of WIN 55,212-2 (10 mg/kg) with AM-251 (0.5 mg/kg) on CST in comparison to vehicle-treatment ($P > 0.05$). Also, Tukey's analysis demonstrated that injection of AM-630 (0.5 mg/kg) could not reverse the anticonvulsant effect of WIN 55,212-2 (10 mg/kg) in coinjection treatment ($P > 0.05$).

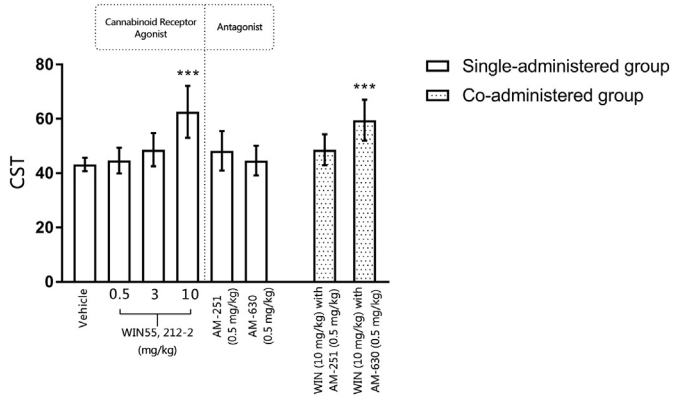

Fig. 1. Effect of administration of WIN 55,212-2 (0.5, 3 and 10 mg/kg), AM-251 (0.5 mg/kg), AM-630 (0.5 mg/kg), and coadministration of WIN 55,212-2 (10 mg/kg) with AM-251/AM-630 on CST. Values are expressed as the mean ± standard error of the mean (S.E.M.), from 10 animals in each group and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$P < 0.001$ compared to vehicle-treated group. Vehicle: saline/DMSO.

3.2. Anticonvulsant role of K-channels

According to one-way ANOVA, administration of K-channel opener (cromakalim at 10 μg/kg) and K-channel blocker (GLI at 0.03 and 1 mg/kg) could not induce a significant effect on CST in comparison with vehicle-treated animals ($F(3, 36) = 3.259$, $P > 0.05$, Fig. 2).

3.3. The collaboration of CBRs and K-channels

Based on one-way ANOVA, cotreatment of WIN 55,212-2 at its subeffective dose (3 mg/kg) with cromakalim (10 μg/kg) induced a significant increase in the CST ($F(11, 108) = 2.244$, $P < 0.001$, Fig. 3) in comparison to single injection of WIN 55,212-2 (3 mg/kg) (postanalysis: $P < 0.05$), and cromakalim (10 μg/kg) (postanalysis: $P < 0.01$). Also, GLI at the higher dose (1 mg/kg) could significantly reverse the effect of WIN 55,212-2 (10 mg/kg) in comparison to treatment of WIN 55,212-2 at 10 mg/kg ($P < 0.05$). Post hoc studies showed that combination of GLI (1 mg/kg) with 10 mg/kg of WIN 55,212-2 significantly decreased the anticonvulsant effect of single treatment of WIN 55,212-2 ($P < 0.001$); moreover, addition of AM-251 to 0.03 mg/kg of GLI and 10 mg/kg of WIN 55,212-2 had no significant effect on CST ($P > 0.05$).

3.4. Assessment of mitochondrial ATP level of the hippocampus

In the final step, we tried to evaluate the ATP level of the hippocampus to clarify the effect of each treatment on ATP concentration. One-way ANOVA revealed that CBR agonist could inhibit the mitochondrial function and significantly decreased ATP level of the hippocampus ($F(10, 44) = 8.354$, $P < 0.001$, Fig. 4). Postanalysis showed that WIN 55,212-2 at the higher dose (10 mg/kg) significantly decrease the ATP level of the hippocampus ($P < 0.01$). To clarify the impact of each CB1 and CB2 receptors on ATP level, we tried to use AM-251 and AM-630 in coadministration with WIN 55,212-2 (10 mg/kg). Analysis indicated that CB2 receptor inhibitor (AM-630) could not significantly neutralize the effect of WIN 55,212-2 ($P > 0.05$), however, AM-251 as a CB1 antagonist could reverse the effect of WIN 55,212-2 (10 mg/kg) in this experiment ($P < 0.05$). On the other hand, none of K channel activator and inhibitor could impact on ATP level ($P > 0.05$). At the final part, to investigate the interaction of WIN 55,212-2 and K channels factors, we use a combination therapy of WIN 55,212-2 at 3 mg/kg (subeffective dose) with cromakalim (10 μg/kg) and GLI (1 mg/kg). Analysis showed that both cromakalim and GLI could not significantly alter the effect of CBR agonist in WIN 55,212-2 (3 mg/kg)-treated animals ($P > 0.05$). Also, combination treatments of WIN 55,212-2 at

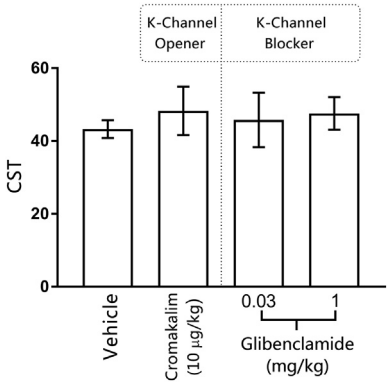

Fig. 2. Effect of administration of cromakalim (10 μg/kg) and GLI (0.03 and 1 mg/kg) on CST. Values are expressed as the mean ± S.E.M., from 10 animals in each group and were analyzed using one-way ANOVA followed by Tukey's post hoc test. Groups were compared to vehicle-treated animals. Vehicle: saline/DMSO.

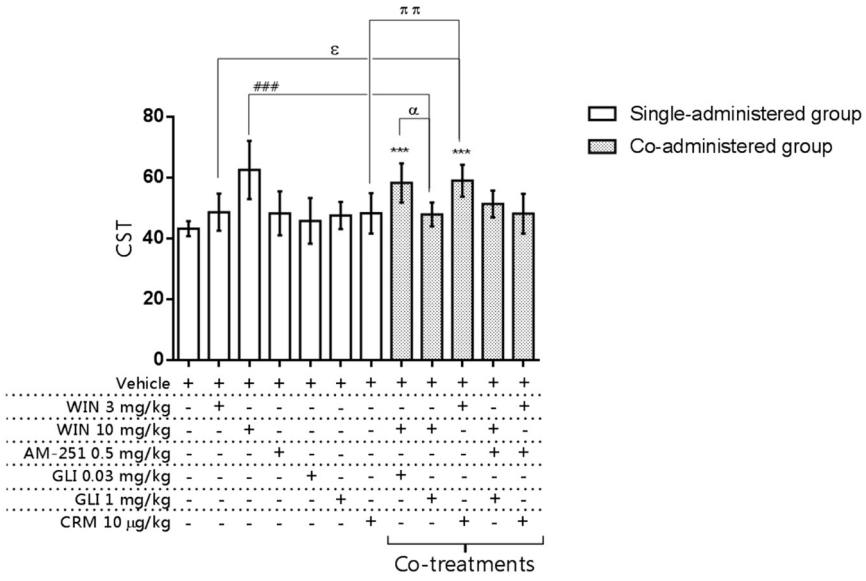

Fig. 3. Coadministration of WIN 55,212-2 (10 mg/kg) with GLI (0.03 and 1 mg/kg), WIN 55,212-2 (10 mg/kg) with cromakalim, WIN 55,212-2 (10 mg/kg) with AM-251, WIN 55,212-2 (10 mg/kg) with AM-251 and GLI (1 mg/kg), and WIN 55,212-2 (3 mg/kg) with AM-251 and cromakalim. Values are expressed as the mean ± S.E.M., from 10 animals in each group and were analyzed using one-way ANOVA followed by Tukey's post hoc test. α $P < 0.05$ compared to cotreated of WIN 55,212-2 (10 mg/kg) with GLI (0.03 mg/kg), π π $P < 0.01$ compared to cromakalim (10 μg/kg), ε $P < 0.05$ compared to WIN 55,212-2 (3 mg/kg), ### $P < 0.001$ compared to WIN 55,212-2 (10 mg/kg) plus GLI (1 mg/kg), and ***$P < 0.001$ compared to vehicle-treated group. Vehicle: saline/DMSO.

10 mg/kg with cromakalim (10 μg/kg) and GLI (1 mg/kg) significantly decreased the mitochondrial ATP level of the hippocampus ($P < 0.01$).

4. Discussion

The results of our current experiments indicated that WIN 55,212-2, a nonselective CBR agonist, exerts an anticonvulsant-like effect in PTZ model of seizure. In this context, we investigated the possible role of K channels in the anticonvulsant-like effect of CBR agonists through mitochondrial function pathway. We found that K_{ATP} channel inhibitors mitigated the anticonvulsant-like effect of WIN 55,212-2; whereas activation of K_{ATP} channels potentiated the anticonvulsant-like effect of WIN 55,212-2. Also, we showed that this effect might be due to the effect of WIN 55, 212-2 on ATP level of the brain cells.

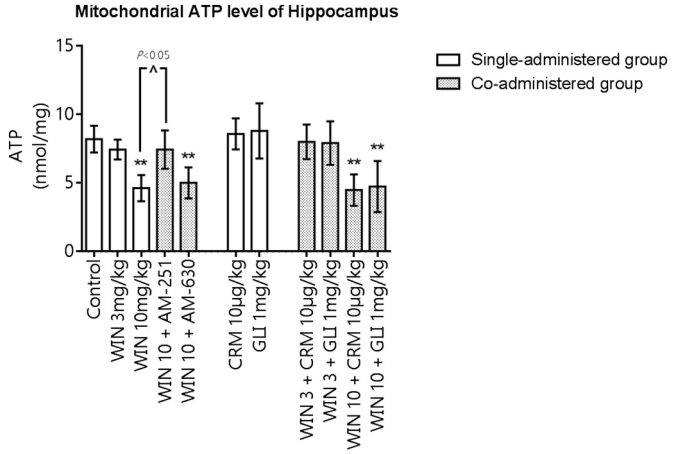

Fig. 4. Effect of WIN 55,212-2 (3 and 10 mg/kg) in combination with AM-251 (0.5 mg/kg) and AM-630 (0.5 mg/kg), GLI (1 mg/kg), and cromakalim (10 μg/kg) on mitochondrial ATP level of the hippocampus. Values are expressed as the mean ± S.E.M., from 5 animals in each group and were analyzed using one-way ANOVA followed by Tukey's post hoc test. **$P < 0.01$ compared to vehicle-treated group (control) and ^$P < 0.05$ compared to WIN 55,212-2 10 mg/kg. Vehicle: saline/DMSO.

Cannabinoid receptors belong to G-protein coupled receptors superfamily, from which CB1 receptor is expressed predominantly in the CNS, existing in copious amounts in the striatum, hippocampus, and cerebellum [32]. It has been well-documented that the endocannabinoid system could have a protective effect against seizure susceptibility in different animal models of epilepsy, including viral encephalopathy and chronic brain injury [33]. In this regard, Rimonabant, a selective CB1 receptor blocker, enhanced the frequency and the duration of pilocarpine-induced seizures [10,34–36]. Our results are consistent with the results of previous studies in which CBR agonists exhibit anticonvulsant-like effects in different models of seizure susceptibility [33]. Pentylenetetrazole model of myoclonic seizure is a valid model for screening the anticonvulsant effect of potential novel drugs, and the PTZ-induced myoclonic seizures are considered as seizures located in the forebrain in correlation with over-activity of the amygdala and piriform cortex [37–39]. We also showed that WIN 55,212-2 at 10 mg/kg significantly increased the CST, implying the capability of this drug to improve the seizure susceptibility. Moreover, our study indicated dose–response effect of WIN 55,212-2 in PTZ model of myoclonic seizures.

Currently, the K_{ATP} channels have been documented to be critically involved in the modulation of seizure susceptibility [40]. In this regard, it has been demonstrated that the administration of cromakalim, a K channel opener, leads to a decreased level of excitability in CA3 hippocampal cells [41]. Also, in animal models of epilepsy, cromakalim has been shown to induce anticonvulsant-like effects. It was also reported that $K_{ir}6.2$-channel and sulphonylurea receptor (SUR1)-two subunits of K_{ATP} channel-knock out mice were more frequently affected by kainic acid-induced seizures [42]. A robust body of in vitro and in vivo pharmacological studies supports this hypothesis that K_{ATP} channels are essential for the modulation of seizure susceptibility [43–48]. Past reports have suggested that K_{ir} could act as a potential antiepileptic drugs, and also some of the known antiepileptic drugs have retrospectively been shown to additionally open K_{ir} channels [16]. Herein, we demonstrated that 10 μg/kg of cromakalim did not significantly alter CST; suggesting this dose as a subeffective dose of cromakalim. Our results also showed that the subeffective dose of cromakalim significantly enhanced the protective effect of WIN 55,212-2 against seizure; indicating that

cannabinoid agonists have a proportional effect through K_{ATP} channels to decrease seizure susceptibility.

Significant importance of cannabinoids in the modulation of K currents in the CNS is well-studied. Indeed, activation of CB1 receptors leads to a decline in cAMP levels [18–21]. In a culture of rat hippocampal neurons, WIN 55,212-2 was shown to induce an alteration in steady-state inactivation of A-type K current around 10 to 20 mV, through the inactivation of cAMP-dependent protein kinase A (PKA) [49,50]. Moreover, cannabinoids are reported to initiate an $K_{ir}6.2$ current [23]. In the current study, both 0.03 and 1 mg/kg of GLI, an antidiabetic drug which blocks both $K_{ir}6.2$/SUR1 subunits of K_{ATP} channels, did not significantly change the seizure threshold. The anticonvulsant effect of WIN (10 mg/kg) was abolished by treatment with 1 mg/kg of GLI. However, this reversal effect was not observed with treatment with 0.03-mg/kg GLI. In addition, our results showed that the cotreatment with an effective dose of WIN and a subeffective dose of AM-251, a specific antagonist for the CB1 receptor, did not cause any significant changes in the anticonvulsant effect of WIN. Notwithstanding, treatment with GLI (0.03 mg/kg) alongside with previous doses of WIN and AM-251 eliminated the anticonvulsant effect of WIN (10 μg/kg). All taken together, these data confirm that cannabinoid agonists play the part of their anticonvulsant role via K_{ATP} channels; while K_{ATP} channel inhibitors reverse these anticonvulsant effects.

Past reports have demonstrated that CBR agonist could inhibit the mitochondrial function by decreasing the ATP level, complex I/II/III activity, and oxygen consumption [11]. It has been reported that the CBRs and K channels like K_{ATP} and $K_{ir}6.2$ channel are in interaction with each other [22,23]. The mechanism of this interaction is completely understood, however, in this manuscript, we investigated that CBR agonist could inhibit the mitochondrial function of the hippocampus and reduce ATP level. This condition could alter the K_{ATP} channel function. According to past studies, low level of ATP could open the K_{ATP} and subsequently decrease the seizure susceptibility [22]. In this study, CBR agonist at its subeffective dose could not significantly decrease ATP level, however, using this treatment in a combination of a K_{ATP} activator could intensify the effect of CBR agonists. Also, we demonstrated that only CB1 receptors are involved to reduce the brain ATP level. According to our results, we hypothesize that the interaction of CBR and K_{ATP} is related to ATP level of neural cells through inhibiting the mitochondria and decreasing in the ATP level by CBR agonist and subsequently this alteration could inhibit K_{ATP}. In this regard, activation of this channel, could promote the anticonvulsant effect of CBR agonists. Also, to clarify the interaction between CBR agonist and K_{ATP}, we use a combination of CBR agonist with K_{ATP} inhibitor at their effective dose; results suggested that K_{ATP} inhibitor could reverse the anticonvulsant effect of CBR agonists. Briefly, CB1 agonist accomplishes at least a part of its anticonvulsant actions through ATP-sensitive K, probably channels by inhibiting the mitochondria and decreases the ATP level to open the K channel to induce its anticonvulsant effect. Therefore, a combination of CB1 receptor agonist and K_{ATP} channels activator may be a novel treatment of epilepsy to reduce the receiving dose of CBR agonists.

Evaluation mitochondrial ATP level of the hippocampus, after pharmacological treatments, is only one part of the puzzle for concluding that the mitochondrial function is the link between K-channel and CBR activity. In this regard, in-vitro evaluation of CBR agonists and antagonists' effects on the behavior of K_{ATP}-channels could improve the investigation of the hypothesis that CBR activity could modulate the K_{ATP}-channel activity through alteration of ATP levels. In this regard, we recommend further studies to evaluate the in-vitro interaction between CBRs and K_{ATP}-channels by using whole-cell patch-clamp. Unfortunately, using this technique is considered as the limitation of our study.

5. Conclusion

In conclusion, we showed that cannabinoid agonists hold anticonvulsant properties in PTZ-induced model of clonic seizure. Also in the current study, we revealed that only CB1 receptor could involve in anticonvulsant effects in mice. Furthermore, we demonstrated that K_{ATP} channels play a pivotal role in the mediation of the anticonvulsant effect of cannabinoids. Also, using K_{ATP} channel activator agents could decrease the dose of CBR agonist to increase the seizure threshold. Also, we observed that CB1 receptor agonist could decrease the mitochondrial ATP level of the hippocampus which might be involved in relation pathway of cannabinoid system and K-channels.

Conflict of interest

All authors declare that there is no conflict of interest.

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