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Role of cannabinoid CB1 receptors in the proconvulsant effect of Apelin-13 on penicillin-induced epileptiform activity

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Epilepsy is a widespread neurological disorder. Many neurotransmitters, neuropeptides and neuromodulators have a significant role in the epileptic activity. Apelin-13 and cannabinoid CB1 receptor agonist and antagonist have an effect in the penicillin model of epilepsy. The relationship between apelin and epilepsy, and the apelin-cannabinoid relationship in epilepsy is still not well understood. Thus, this study focuses on the relationship between apelin-13 and CB1 receptor in experimental model of epilepsy. Penicillin injection was given intracortically (i.c.) for the development of epileptic seizures. Ninety-one male Wistar rats were divided into 13 groups. CB1 receptor agonist ACEA (7.5 µg, intracerebroventricularly, icv) and antagonist AM-251 (0.25 µg and 0.125 µg, icv) were administered to three different groups, two different doses of apelin-13 (5 µg and 15 µg, icv) were applied and the interactions between these five groups of substances were evaluated. Both apelin-13 (15 µg) and AM-251 (0.25 µg) raised the spike frequency of epileptiform activity separately. Application of apelin-13 + AM-251 also increased the spike frequency of epileptiform activity beginning in the 30 min after apelin-13 application. When the non-effective dose of AM-251 and the effective dose of apelin-13 were administered together, epileptic activity increased in the 20 min. ACEA reduced the epileptiform activity starting in the 50th min. apelin-13 and ACEA administration in effective doses decreased epileptiform activity. The non-effective doses of AM-251, apelin-13 and effective dose of ACEA decreased the epileptiform activity in the 50 min. Application of non-effective doses of apelin and AM-251 together does not induce any additional proconvulsant activity, and CB1 receptor agonist, ACEA reversed the proconvulsant activity of apelin-13. These results suggest that they utilize different receptors to begin their own effects by increasing intracellular Ca^{2+} in epilepsy. Considering that apelin-13 is an endogenous substance known for its neuroprotective properties, the proconvulsant effect of apelin-13 in the presented study is remarkable.

Keywords: Brain electrocorticograhy, Cannabinoid CB1 receptor agonists, Epilepsy, Neuromodulators

Epilepsy is one of the most common neurological disorders characterized by recurrent seizures in the brain[1]. Epilepsy is a chronic noncommunicable disease of the brain that affects around 50 million people worldwide according to World Healt Organization (WHO)[2]. Apelin, a neuropeptide, firstly was isolated from a bovine stomach by Tatemoto *et al*.[3] after apelin receptor was found in 1993[4]. It is a typical endogenous ligand for G protein-coupled apelin receptor (APJ)[5]. Apelin gene that is produced as 77 amino acid preproapelin has subtypes of apelin-13, -16, -17, -19 and -36[6]. Apelin-13 has the most powerful bioactivity and receptor binding capacity, also has a lot of biological functions[7]. Apelin mRNAs, proteins and APJ are commonly found in peripheral tissues and the central nervous system (CNS) and especially in the cerebellum, hypothalamus, lung, heart, kidney and endothelium[8]. Hence, apelin-APJ system may be related to many physiological and pathological processes such as neuroprotection[9]. However, there are few studies showing apelin and epilepsy interactions. In literature, apelin-13 decreased the incidence of PTZ-induced seizure in rats and appeared preservative effects and decreased markers of cell injury and death in primary cortical glia-neuron co-culture of rat against PTZ-induced toxicity through its calcium blocking, antioxidant, anti-apoptotic and anti-inflammatory properties[10,11]. Other study demonstrated protective effects of apelin against neuronal death in epilepsy both *in vitro* and *in vivo*[12]. Conversely, apelin-13 increased the spike frequency of penicillin-induced epileptiform activity in rat[13].

The cannabinoid system has also a notable role in neurophysiological and pathological processes including epilepsy. The CB1 receptor mediates many of the anticonvulsant effects of cannabinoids[14] and has a significant function in regulating synaptic transmission[15]. WIN 55,212-2, CB1 receptor agonist,

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completely suppressed status epilepticus (SE) activity in the pilocarpine model, the neuronal culture-induced epilepsy model and spontaneous recurrent epileptiform discharges with CB1 receptor activity and increased penicillin-induced epileptiform activity in rat[16]. Application of AM-251 (CB1 receptor antagonist) inhibited the anticonvulsant effect of WIN55,212-2[17]. On the other hand, high activation of N-methyl-D-aspartate (NMDA) receptor is related with a variable number of neurological disorders including epilepsy[18].Interestingly, apelin-13 and CB1 receptors have relationship with NMDA receptor activity[10,19,20]. The apelin-APJ organization has been showed as a neuroprotective modulator for cortical and hippocampal neurons against NMDA receptor mediated neuronal injury[4]. Apelin showed neuro-protective effect by induction of Ca^{2+} transient with attenuation of NMDA receptor-mediated Ca^{2+} accumulation[21]. WIN55,212-2 dose-dependently diminished both cell death and Ca^{2+} flux induced by NMDA receptors in both cultured neuronal cell border and primed dorsal root ganglion neurons[22]. A relationship between memantine (NMDA receptor antagonist) and AM-251 in penicillin-induced epileptiform activity in rats has been reported[23].

Since there are a few controversial reports about the effect of apelin-13 on epileptic activity and also there is no investigation to evaluate the interaction between apelin-13 and cannabinoid CB1 receptor in epilepsy, in the present study, we explored the role of CB1 receptor antagonist and agonist in the proconvulsant effect of apelin-13 on the penicillin-induced epileptiform activity in rat.

Materials and Methods

Animals

Ninety-one male Wistar rats (4 months) were obtained from the Experimental Animal Application and Research Center of the University of Ondokuz Mayis, Samsun, Turkey. Animals weighing 180-270 g were housed in a temperature and humidity controlled conditions on a 12 h dark/light cycle with free access to standard lab food and water. Local Ethics Committee for Animals Experiments of Ondokuz Mayis University confirmed all experimental procedures (2014/46). Experimental groups were randomly divided into 13 groups as follows: Gr. I: No treatment (control); Gr. II: experimental control with 2.5 µL saline (ic); Gr. III: Penicillin-G (PEN) (2.5 µL, ic); Gr. IV: PEN (2.5 µL, ic) + 15 µg of Apelin-13 (icv); Gr. V.:PEN (2.5 µL, ic) + 5 µg of Apelin-13 (icv); Gr. VI: PEN (2.5 µL, ic) +7.5 µg ACEA (icv); Gr. VII-VIII: PEN (2.5 µL, i.c.) +0.25 and 0.125 µg AM-251 (icv), respectively; Gr. IX: PEN (2.5 µL, ic) +0.25 µg AM-251 (icv) + 15 µg Apelin-13 (icv); Gr. X: PEN (2.5 µL, ic) + 7.5 µg ACEA (icv) + 15 µg Apelin-13 (icv); Gr. XI: PEN (2.5 µL, ic) + 0.125 µg AM-251 (icv) + 15 µg Apelin-13 (icv); Gr. XII: PEN (2.5 µL, ic) + 0.125 µg AM-251 (icv) + 5 µg Apelin-13 (icv); and Gr. XIII: PEN (2.5 µL, ic) + 0.125 µg AM-251 (icv) + 5 µg Apelin-13 (icv) + 7.5 µg ACEA (icv). Each group had 7 rats.

Electrodes placement for electrocorticography (ECoG) recordings

Before placed in a rat stereotaxic device, the animals were anesthetized with urethane (1.25 g/kg, i.p.). Two screw electrodes were located over the left somatomotor cortex stereotaxically. Bipolar two Ag–AgCl ball electrodes were located over the left somatomotor cortex. For intracortical injections for the left somatomotor cortex, urethane anesthetized (intraperitoneal injection, i.p.) rat skull was opened holes with a hand drill in the bone with the coordinates of 2 mm lateral to the midline, 2 mm posterior to the bregma and 3.5 mm ventral to the surface of the skull[24]. Icv injections were applied into the rats left lateral ventricle, with the coordinates of 1.1 mm lateral to the midline, 1.5 mm posterior to the bregma and 4.2 mm ventral to the surface of the skull. First drug administrated 30 min after penicillin injection. After this other drugs administrated to through 10 min interval. The electrocorticographic (ECoG) activity was continously monitored (PowerLab, 4/SP, AD Instruments, Castle Hill, NSW, Australia). Eventually, the amplitude and spike frequency of the epileptiform ECoG activity were examined off-line.

Substance administration

AM-251 (N-(piperidine-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1Hpyrazole-3-carbo-xamide) and ACEA (arachidonyl-2-chloroethylamide) (Sigma Chemical Co.) were purchased for experiments. ACEA and AM-251 were dissolved in dimethylsulfoxide (DMSO) with saline (DMSO/saline 3:7 volume/volume), and the necessary doses were administered icv in 2 µL. AM-251 and ACEA dosages were decided as per Kozan *et al*.[25]. Apelin-13 ($C_{69}H_{111}N_{23}O_{16}S \cdot C_2F_3O_2$) purchased Sigma. Apelin-13 was dissolved in distilled water and its doses were injected icv in a volume of 2 µL. Apelin-13 doses determined according to a previous study conducted

in our laboratory[26]. About 500 IU penicillin-G was dissolved in distilled water and injected i.c. in a volume of 2.5 µL.

Statistical analysis

After all recorded electrophysiological data converted to numeric values utilizing SPSS (Statistical Package for the Social Sciences) software through 17.0. Data were eveluated in 10 min periods for three hours after the drugs injections. All values are shown as means i.e., standard error of the mean (SEM). The distribution of the data of was determined using the Shapiro-Wilk test. For multiple comparisons, one-way analysis of variance (ANOVA) and Tukey-Kramer post-hoc test were performed to determine differences between the groups. In all experimental groups P <0.05 were statistically significant. The percent frequency of epileptiform ECoG activity value is defined as: the mean of spike frequency after substance administered / the mean of spike frequency before substance administered × 100.

Results

To induce epileptiform activity, after penicillin-G application, spike wave complexes were observed in the 2-5 min. Epileptic activity stabilized 30 min after penicillin administration and continued for 3 h. The means of spike frequency and amplitude of epileptiform activity were 41.4±2.2 spike/min and 1000±82 µV in the 90 min after the penicillin-G administration, respectively (Suppl. Fig. S1A). [*All supplementary data are available only online along with the respective paper at the journal website* (*http://ijeb.res.in*) *as well as NOPR repository at http://nopr.res.in*].

Effect of apelin on penicillin-induced epileptiform activity

The non-effective and effective doses of apelin-13 (5 and 15 µg) were taken as reference in this study[7]. Apelin-13 @5 µg did not change epileptiform activity (Fig. 1) (Gr. V). The means of spike frequency and amplitude of epileptiform activity were 47.2±5.2 spike/min and 907±83 µV in the 90 min after 5 µg apelin-13, respectively (Suppl. Fig. S1B). Apelin-13 (15 µg) significantly increased spike frequency of epileptiform activity compared to the penicillin group without altering amplitude (Fig. 1) (Gr. IV). The means of spike frequency and amplitude of epileptiform activity were 61.9±5.6 spike/min and 1044±121 µV in the 90 min after 15 µg apelin-13 administration, respectively (Suppl. Fig. S1C).

ACEA and AM-251's effect on penicillin-induced epileptiform activity

The non-effective dose of AM-251 (0.125 µg) didn't change either spike frequency or amplitude of epileptiform activity (Gr. VIII), while the effective dose AM-251 (0.25 µg) caused proconvulsant activity in the 20 min after AM-251 injection by increasing the spike frequency of epileptiform activity (Fig. 2)

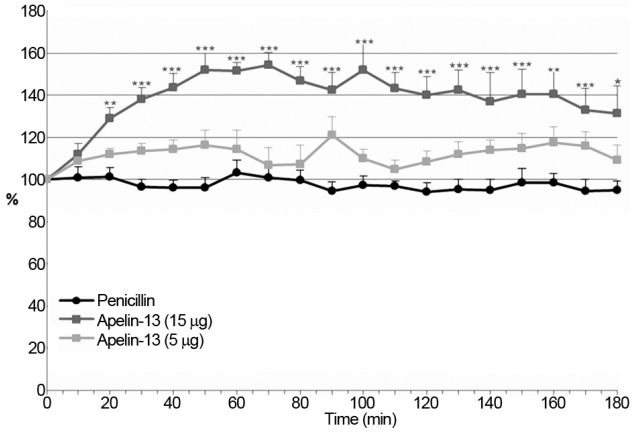

Fig. 1 — Effect of icv application of the effective and non-effective doses of apelin-13 on the mean spike frequency of penicillin-induced epileptiform activity. Apelin-13 5 µg did not alter epileptiform activity, whereas 15 µg it significantly raised the mean spike frequency of the epileptiform activity in the 20th min without altering the amplitude. [Two groups were compared by Independent-Samples t-test. P <0.05; **P <0.01; ***P <0.001 show significant differences compared to penicillin group]

$$\text{Frequency value \%} = \frac{\text{Mean spike frequency after substance administred}}{\text{Mean spike frequency before substrance administred}} \times 100$$

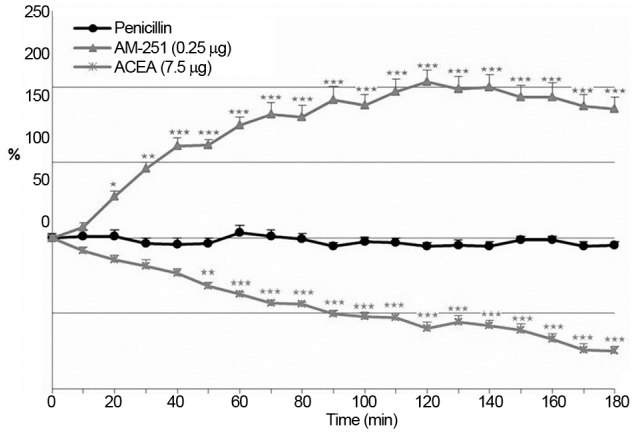

Fig. 2 — Effect of icv application of ACEA and AM-251 on the mean spike frequency of penicillin-induced epileptiform activity. ACEA 7.5 µg decreased the spike frequency of epileptiform activity in the 50th min without altering the amplitude, and 0.25 µg AM-251 caused proconvulsant activity in the 20th min. [Two groups were compared by Independent-Samples t-test. *P <0.05; **P <0.01; ***P <0.001 show significant differences compared to penicillin group]

$$\text{Frequency value \%} = \frac{\text{Mean spike frequency after substance administred}}{\text{Mean spike frequency before substrance administred}} \times 100$$

(Gr. VII). The means of spike frequency and amplitude of epileptiform activity were 47.4±3.5, 82.3±8.4 spike/min and 930±72, 1067±104 μV in non-effective (0.125 μg) and effective (0.25 μg) doses of AM-251, respectively (Suppl. Fig. S1 D-E). AM-251 (0.25 μg) also induced SE-like activity.

ACEA (7.5 μg, effective dose,) reduced the spike frequency of epileptiform activity in the 50 min after ACEA administration without changing the amplitude (Fig. 2) (Gr. VI). The means of spike frequency and amplitude of epileptiform activity were 21.0±2.2 spike/min and 839±106 μV in the 90 min after the ACEA administration, respectively Suppl. Fig. S1F).

Interaction between apelin and CB1 receptor

Apelin-13 (15 μg) was administered 10 min after 0.25 μg AM-251 injection. Application of 0.25 μg AM-251 + 15 μg apelin-13 induced epileptic activity starting in the 30 min after apelin-13 administration (Fig. 3) (Gr. IX). The means of spike frequency and amplitude of epileptiform activity were 86.1±8.0 spike/min and 1069±56 μV in the 90 min after the AM-251 administration in this group, respectively (Suppl. Fig. S2A).

Application of 7.5 μg ACEA 10 min before 15 μg apelin-13 injection reduced the spike frequency of epileptiform activity in the 40 min, without altering the amplitude (Fig. 3) (Gr. X). The means of spike frequency and amplitude of epileptiform activity were 30.0±8.4 spike/min and 700±82 μV in the 90 min after the ACEA administration, respectively (Suppl. Fig. S2B).

Administration of AM-251 (0.125 μg) did not effect proconvulsant activity of apelin-13 (15 μg) (Gr. XI). The proconvulsant activity was observed in the 30 min after AM-251 application (Fig. 2). The means of spike frequency and amplitude of epileptiform activity were 61.3±3.6 spike/min and 849±50 μV in the 90 min after the AM-251 administration, respectively (Suppl. Fig. S2C).

The application of non-effective doses of AM-251 (0.125 μg) + Apelin-13 (5 μg) did not change epileptiform activity (Fig. 3) (Gr. XII). The means of spike frequency and amplitude of epileptiform activity were 53.2±8.1 spike/min and 1018±102 μV in the 90 min after the AM-251 administration, respectively (Suppl. Fig. S2D). No synergistic effect was observed between AM-251 and apelin-13.

In this study, the relationship between AM-251 and ACEA with the proconvulsant effect of apelin-13 was investigated. AM-251 (0.125 μg), apelin-13 (5 μg) and ACEA (7.5 μg) (Gr. XIII) diminished the epileptiform activity in the 50 min after apelin-13 administration (Fig. 3). The means of spike frequency and amplitude of epileptiform activity were 31.5±7.0 spike/min and 987±89 μV in the 90 min after the AM-251 administration, respectively (Suppl Fig. S2E). We showed that apelin-13 and CB1 receptor antagonist AM-251 have proconvulsant activity, while CB1 receptor agonist ACEA has anticonvulsant activity in penicillin-induced epilepsy. Since synergistic effect did not occur we assume that there is no relationship between apelinergic and cannabinoidergic systems.

Discussion

In this study, we investigated the role of cannabinoid CB1 receptor in the proconvulsant effect of apelin-13

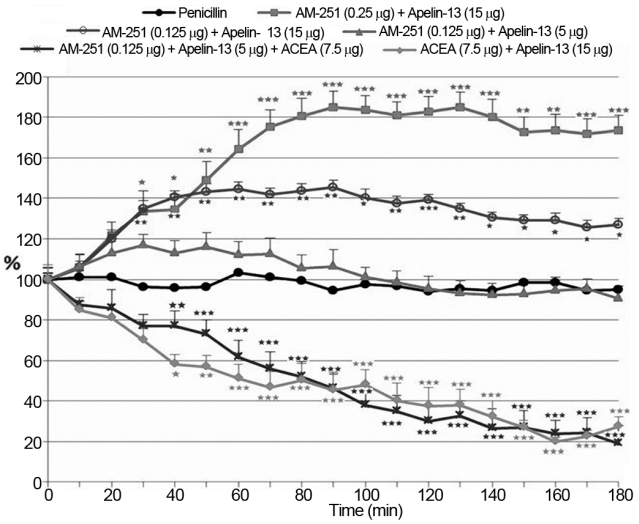

Fig. 3 — Effect of icv administration of AM-251 (0.25 & 0.125 μg), ACEA (7.5 μg) and apelin-13 (5 & 15 μg), on the mean spike frequency of penicillin-induced epileptiform activity. Administration of apelin-13 (15 μg) 10 min after AM-251 (0.25 μg) caused an anticonvulsant effect on the mean spike frequency of epileptiform activity within 30 min of the apelin-13 administration. However, administration of apelin-13 (15 μg) 10 min after 0.125 μg AM-251 did not change proconvulsant effect of apelin-13. The proconvulsant activity emerged in the 30 min after AM-251 administration. AM-251 @0.125 μg and 5 μg apelin-13 did not affect the mean spike frequency of epileptiform activity. Application of an effective dose of ACEA (7.5 μg) 10 min before apelin-13 (15 μg) injection declined the spike frequency of epileptiform activity in 40th min, but did not change the amplitude. AM-251 @0.125 μg, 5 μg apelin-13 and 7.5 μg ACEA decreased the epileptiform activity in the 50 min after apelin-13 administration. [More than two groups were compared by one-way ANOVA followed by Tukey post hoc test. **P* <0.05; ***P* <0.01; ****P* <0.001 show significant differences compared to the penicillin group]

$$\text{Frequency value \%} = \frac{\text{Mean spike frequency after substance administred}}{\text{Mean spike frequency before substrance administred}} \times 100$$

on the penicillin-induced epileptiform activity. The doses of CB1 receptor agonist ACEA and CB1 receptor antagonist AM-251[25] and apelin-13[26] were based on previous work done in our laboratory. Apelin increased the spike frequency of seizures, and AM-251 caused status epilepticus-like activity whereas ACEA showed protective effect against epileptic activity.

The apelin/APJ system has neuroprotective effect[9,27], including anti-inflammatory, anti-oxidative stress, anti- apoptosis, and regulating autophagy, blocking excitatory toxicity[28]. Apelin inhibited NMDA receptor-mediated excitotoxic signal cascades, as well as enhances neuronal survival[22]. For this reason, the endogenous expression of apelin in neurons and the apelin-APJ interaction are thought to be a neuroprotective signal transduction in the CNS. In kainic acide-induced status epilepticus (SE), APJ activation reduced epileptic activity, the apelin-APJ system modulated postsynaptic currents mediated by NMDA receptor compare to patch-clamp recordings[7]. In addition to reports demonstrating apelin's neuroprotective properties, there are few studies in the literature showing apelin and epilepsy associations. Two of these studies examined apelin expression and apelin level in the plasma. Apelin expression was studied in the temporal cortex of temporal lobe epilepsy patients and hippocampus and cortex structures in the experimental pilocarpin-induced experimental epilepsy model[29]. Plasma apelin levels in children with idiopathic generalized epilepsy treated with valproate were higher than control group[30]. However, the cause of the increase in both expression and plasma levels of apelin is unknown. Apelin-13 had proconvulsant effect in the model of penicillin-induced epileptiform activity according to the study carried out in our laboratory[26]. In hippocampal neurons, apelin-APJ signaling represents a significant endogenous pro-survival pathway[9]. Apelin-13 decreased the incidence of seizure in the PTZ-induced seizure model in rats[10]. In contrast, apelin-13 shows proconvulsant effect in the penicillin-induced epileptic activity in the present study. The endogenous apelin is up-regulated in the epileptic human and rat brain[29]. There has been no assessment until now to clarify whether this increment is a harmful mechanism following seizure or a protective way to declining post-seizure injury[10]. The reason for this discrepancy may be due to the different epilepsy model used. However, the roles of endogenous apelin in the CNS are still unclear[31]. In cerebrocortical neurons, apelin-36 inhibits NMDA-mediated Ca^{2+} accumulation, while it does not affect Ca^{2+} accumulation induced by K^{+} depolarization[21]. G protein-coupled receptor ligands can trigger neuronal Ca^{2+} transmission[32]. Likewise, in cerebrocortical neurons apelin also induces Ca^{2+} transition in a dose-dependent manner[21]. Apelin isoforms (13, 17 and 36) have been shown to increase intracellular Ca^{2+} levels in human NT2.N neurons[33]. Based on this, apelin may have a proconvulsant effect on epilepsy by increasing intracellular calcium.

Cannabinoids are known to suppress seizures. Cannabinoids show an anticonvulsant effect by different pathways. In anticonvulsant and neuro-protective effects of cannabidiol, the modulation of PI3K/mTOR signaling pathway is involved[34]. Although CB1 receptors are located largely on a type of GABAergic interneuron nerve terminal in neocortex, amygdala, and hippocampus, in another area of brain, they are mostly found on the excitatory terminals of glutamatergic system[35]. The cannabinoid system is involved in the regulation of GABA and glutamate release depending on the brain region involved[36]. In addition to this cannabinoids also block GABA reuptake in the globus pallidus[37]. Cannabinoid receptors inhibit adenylyl cyclase (AC) and protein kinase A (PKA), regulate the activation of mitogen-activated protein kinases (MAPKs) which includes extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 kinases, by Gi/o signaling[38]. Intracerebroventricular application of AM-251 raised the spike frequency of penicillin-induced epileptiform activity in rat, whereas ACEA suppressed the spike frequency of epileptiform activity[25]. While the CB1 receptor agonist ACEA (7.5 μg, icv) displayed an anticonvulsant effect, the CB1 receptor antagonist AM-251 (0.5 μg, icv) exhibited a proconvulsant effect in pentylenetetrazol model of epilepsy in rats[36]. AM-251 administration resulted in the development of SE-like activity characterized by bursting and spiking activity[16]. While AM-251 intensified proconvulsant effect of Toxoplasmosis, ACEA inhibited proconvulsant effect of Toxoplasmosis in mice[39]. Doses of 2 and 8 mg/kg specific cannabinoid CB1 agonist ACEA had shown anticonvulsant effect against PTZ-induced myoclonic seizures[14]. The results of present study confirm previous studies considering the effects of ACEA and AM-251. Moreover, the relationship between apelin-

13 and cannabinoid CB1 receptor was investigated in the penicillin-induced epilepsy model. Administration of AM-251 and apelin-13 together caused a statistically significant raise in the spike frequency compare to the penicillin group but additional increment was not seen in the spike frequency of epileptic activity compared to AM-251 and apelin group alone. However, CB1 receptor agonist ACEA reversed proconvulsant activity of apelin. While apelin-13 increases intracellular calcium via the APJ receptor, ACEA reduces both intracellular calcium via CB1 receptors and provides gene expression by MAPK activation[21,40]. It is reasonable to assume an inhibiton of apelin's proconvulsant activity in the presence of ACEA.

Non-effective doses of AM-251 + Apelin-13 did not show any effect on epileptiform activity when they were administered together. The Ca^{2+} input caused by both pathways is not enough to cause raise in the spike frequency of epileptic activity. Since non-effective doses of apelin-13 and AM-251 were administered together which did not have a synergistic effect, suggesting substances use their own pathways separately.

A statistically significant decrease in the spike frequency was observed after the administration of AM-251+Apelin-13+ACEA. This reduction is equivalent to the anticonvulsant effect of ACEA. At that case, it might be assumed that CB1 receptor agonists, ACEA, inhibit protein kinase A by decreasing cAMP production through activation of inhibitor G proteins[41], which reduces the amount of intracellular Ca^{+2} through voltage-gated Ca^{2+} channels, resulting in a reduction in the presynaptic transmitter release[13].

Conclusion

The above results demonstrated that the neuropeptide apelin-13 enhances the spike frequency of penicillin-induced epileptiform activity, which suggests a proconvulsant action in the experimental epilepsy model. Further, Cannabinoid CB1 receptor agonist ACEA reduced the spike frequency of penicillin-induced epileptiform activity without changing amplitude, whereas AM-251 enhanced the same. Apelin and AM-251 in a combination with non-effective doses did not lead to the proconvulsant activity. Overall, the electrophysiological data of the present study reveal that apelin-13 has proconvulsant effect on penicillin-induced epileptiform activity and there is no direct interaction between cannabinoid CB1 receptor and apelin-13. Both systems (CB1 receptor and APJ) use intracellular Ca^{2+}. However intracellular pathways of both systems need to be explored in epilepsy.

Conflict of Interest

Authors declare no competing interests.

References

1 Yildiz A, Kocacan SE & Him A, Antiepileptic effects of exenatide in penicillin induced acute epilepsy model in rats, *Indian J Exp Biol*, 61 (2023) 417.

2 World Health Organization. Epilepsy. Published online 2023. https://www.who.int/news-room/fact-sheets/detail/epilepsy (Accessed on 24 October 2023).

3 Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H & Fujino M, Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun*, 251 (1998) 471.

4 O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL, Shi X, Petronis A, George SR & Nguyen T, A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene*, 136, (1993) 355.

5 Wang X, Zhang L, Li P, Zheng Y, Yang Y & Ji S, Apelin/APJ system in inflammation. *Int İmmunopharmacol*, 109 (2022) 108822.

6 Cheng B, Chen J, Bai B & Xin Q, Neuroprotection of Apelin and its signaling pathway. *Peptides*, 37 (2012) 171.

7 Zhang Y, Jiang W, Sun W, Guo W, Xia B, Shen X, Fu M, Wan T & Yuan M, Neuroprotective Roles of Apelin-13 in Neurological Diseases. *Neurochem Res*, 48 (2023) 1648.

8 Yanjie Yang, Shuang-Yu Lv, Shuang-Kun Lyu, Dongdong Wu & Qiang Chen, The protective effect of Apelin on ischemia/reperfusion injury. *Peptides*, 63 (2015) 43.

9 O'Donnell LA, Agrawal A, Sabnekar P, Dichter MA, Lynch DR & Kolson DL, Apelin, an endogenous neuronal peptide, protects hippocampal neurons against excitotoxic injury. *J Neurochem*, 102 (2007) 1905.

10 Kalantaripour TP, Esmaeili-Mahani S, Sheibani V, Asadi-Shekaari M & Pasban-Aliabadi H, Anticonvulsant and neuroprotective effects of Apelin-13 on pentylenetetrazole-induced seizures in male rats. *Biomed Pharmacother*, 84 (2016) 258.

11 Kalantaripour TP, Esmaeili-Mahani S, Sheibani V, Najafipour H & Asadi-Shekaari MM, Apelin-13 protects rat primary cortical glia-neuron co-culture against pentylenetetrazole-induced toxicity. *Biomed Pharmacother*, 87 (2017) 661.

12 Dong H, Dong B, Zhang N, Liu S & Zhao H, microRNA-182 Negatively Influences the Neuroprotective Effect of Apelin Against Neuronal Injury in Epilepsy. *Neuropsychiatr Dis Treat*, 16 (2020) 327.

13 Mackie K, Devane WA & Hille B, Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol Pharmacol*, 44 (1993) 498.

14 Wallace MJ, Wiley JL, Martin BR & DeLorenzo RJ, Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *Eur J Pharmacol*, 428 (2001) 51.

15 Schlicker E & Kathmann M, Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci*, 22 (2001) 565.
16 Arslan G, Alici SK, Ayyildiz M & Agar E, The role of CB1-receptors in the proconvulsant effect of leptin on penicillin-induced epileptiform activity in rats. *CNS Neurosci Ther*, 19 (2013) 222.
17 Nakatsuka T, Chen HX, Roper SN & Gu JG, Cannabinoid receptor-1 activation suppresses inhibitory synaptic activity in human dentate gyrus. *Neuropharmacology*, 45 (2003) 116.
18 Xu XX & Luo JH, Mutations of N-Methyl-D-Aspartate Receptor Subunits in Epilepsy. *Neurosci Bull*, 34 (2018) 549.
19 Luo J, Zhao Q, Li Z & Chen L, Multiple roles of apelin/APJ system in eye diseases. *Peptides*, 152 (2022) 170767.
20 Rivas-Santisteban R, Lillo A, Lillo J, Rebassa JB, Contestí JS, Saura CA, Franco R & Navarro G, N-Methyl-D-aspartate (NMDA) and cannabinoid CB_2 receptors form functional complexes in cells of the central nervous system: insights into the therapeutic potential of neuronal and microglial NMDA receptors. *Alzheimers Res Ther*, 13 (2021) 184.
21 Cook DR, Gleichman AJ, Cross SA, Doshi S, Ho W, Jordan-Sciutto KL, Lynch DR & Kolson DL, NMDA receptor modulation by the neuropeptide Apelin: implications for excitotoxic injury. *J Neurochem*, 118 (2011) 1113.
22 Liu Q, Bhat M, Bowen WD & Cheng J, Signaling pathways from cannabinoid receptor-1 activation to inhibition of N-methyl-D-aspartic acid mediated calcium influx and neurotoxicity in dorsal root ganglion neurons. *J Pharmacol Exp Ther*, 331 (2009) 1062.
23 Cakil D, Yildirim M, Ayyildiz M & Agar E, The effect of co-administration of the NMDA blocker with agonist and antagonist of CB1-receptor on penicillin-induced epileptiform activity in rats. *Epilepsy Res*, 93 (2011) 128.
24 Paxinos G & Watson C, The Rat Brain in Stereotaxic Coordinates. *Academic Press*, New York (1986).
25 Kozan R, Ayyildiz M & Agar E, The effects of intra-cerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. *Epilepsia*, 50 (2009) 1760.
26 Ucar D, The Effect of Apelin-13 on Penicillin-Induced Epileptiform Activity in Rats, Poster Communications. *Acta Physiol*, 215 (2015) 32.
27 Wan T, Fu M, Jiang Y, Jiang W, Li P & Zhou S, Research Progress on Mechanism of Neuroprotective Roles of Apelin-13 in Prevention and Treatment of Alzheimer's disease. *Neurochem Res*, 47 (2022) 205.
28 Zhou JX, Shuai NN, Wang B, Jin X, Kuang X & Tian SW, Neuroprotective gain of Apelin/APJ system. *Neuropeptides*, 87 (2021) 102131.
29 Zhang X, Peng X, Fang M, Zhou C, Zhao F, Zhang Y, Xu Y, Zhu Q, Luo J, Chen G & Wang X, Up-regulation of Apelin in brain tissue of patients with epilepsy and an epileptic rat model. *Peptides*, 32 (2011) 1793.
30 Meral C, Cekmez F, Vurucu S, Tascılar E, Pirgon O, Canpolat FE, Ipcioglu OM, Aydemir G & Aydınoz S, New adipocytokines (vaspin, Apelin, visfatin, adiponectin) levels in children treated with valproic acid. *Eur Cytokine Netw*, 22 (2011) 118.
31 Lv SY, Chen WD & Wang YD, The Apelin/APJ System in Psychosis and Neuropathy. *Front Pharmacol*, 11 (2020) 320.
32 Deiva K, Geeraerts T, Salim H, Leclerc P, Héry C, Hugel B, Freyssinet JM & Tardieu M, Fractalkine reduces N-methyl-d-aspartate-induced calcium flux and apoptosis in human neurons through extracellular signal-regulated kinase activation. *Eur J Neurosci*, 20 (2004) 3222.
33 Choe W, Albright A, Sulcove J, Jaffer S, Hesselgesser J, Lavi E, Crino P & Kolson DL, Functional expression of the seven-transmembrane HIV-1 co-receptor APJ in neural cells. *J Neurovirol*, 6 (2000) S61.
34 Lima IVA, Bellozi PMQ, Batista EM, Vilela LR, Brandão IL, Ribeiro FM, Moraes MFD, Moreira FA & de Oliveira ACP, Cannabidiol anticonvulsant effect is mediated by the PI3Kγ pathway. *Neuropharmacology*, 1 (2020) 108156.
35 Freund TF, Katona I & Piomelli D, Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev*, 83 (2003) 1017.
36 Al-Kaleel A, Aygun H, Al-Gailani L, Kabak Y, Inal S, Ayyildiz M, Him A & Agar E, The electrophysiological and behavioral evaluation of the peptide hemopressin and cannabinoid CB1 receptor agonist and antagonist in pentylenetetrazol model of epilepsy in rats. *Pflugers Arch*, 475 (2023) 719.
37 Sieradzan KA, Fox SH, Hill M, Dick JP, Crossman AR & Brotchie JM, Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology*, 57 (2001) 2108.
38 Tadijan A, Vlašić I, Vlainić J, Đikić D, Oršolić N & Jazvinšćak Jembrek M, Intracellular Molecular Targets and Signaling Pathways Involved in Antioxidative and Neuroprotective Effects of Cannabinoids in Neurodegenerative Conditions. *Antioxidants*, 11 (2022) 2049.
39 Ghanbari MM, Joneidi M, Kiani B, Babaie J & Sayyah M, Cannabinoid receptors and the proconvulsant effect of toxoplasmosis in mice. *Microb Pathog*, 144 (2020) 104204.
40 Howlett AC & Mukhopadhyay S, Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem Phys Lipids*, 108 (2000) 53.
41 Bidaut-Russell M, Devane WA & Howlett AC, Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain. *J Neurochem*, 55 (1990) 21.