RESEARCH PAPER

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Primary somatosensory cortex CB_1 and 5-HT_{1A} receptors interaction in the penicillin model of epilepsy

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Cannabinoid and serotonin systems regulate many biological processes. The aim of the present study was to investigate the functional interaction between the cannabinoid and serotonergic systems of the primary somatosensory region (S1) of the brain in epileptiform activity caused by penicillin. The ACEA (an agonist of CB_1 receptor), AM-251 (an antagonist of CB_1 receptor), 8-OH-DPAT (an agonist of 5-HT_{1A} receptor) and WAY-100635 (an antagonist of 5-HT_{1A} receptor) were administered into the S1 after the same site administration of penicillin in urethane-anesthetized rats. Electrocorticographic recording was done for a 90-min period. The spike waves number and amplitude were recorded in 15-min intervals. Areas under the curve (AUC) of the above-mentioned spike alterations was calculated in 90 min. Spike waves with frequency of 30/min and amplitude of 1.3 mV were appeared after penicillin microinjection. The ACEA (50 ng), 8-OH-DPAT (500 ng) and ACEA (10 ng) plus 8-OH-DPAT (100 ng) reduced epileptiform activity. The AM-251 (50 ng) and WAY-100365 (500 ng) prevented the reducing effects of ACEA (50 ng) and 8-OH-DPAT (500 ng). The AM-251 alone increased spike waves frequency. The AUC results supported the effects of the above-mentioned treatments. The results showed that activating CB_1 and 5-HT_{1A} receptors in the S1 may reduce the epileptiform activity caused by penicillin. Therefore, alone and together activation of central CB_1 and 5-HT_{1A} receptors might be considered in the management of epilepsy treatment.

Key words: cannabinoid, serotonin, primary somatosensory cortex, penicillin, epileptiform activity

INTRODUCTION

Epilepsy is defined as an important neurological disorder resulting from abnormal electrical activity of neurons in various brain structures including cerebral cortex, cerebellum, thalamus and hippocampus (Goodman & Szaflarski, 2021). This abnormal activity can arise from an imbalance between inhibitory gamma-amino butyric acid (GABA), and excitatory (glutamate) neurotransmitter release, expression and function (Wanleenuwat et al., 2020; Akyuz et al., 2021; Sarlo & Holton, 2021). In this context, binding sites of N-methyl-D-aspartate (NMDA) were increased by pentylenetetrazole (PTZ) in the cerebral cortex and hippocampus (Cremer et al., 2009). In addition, $GABA_A$ and $GABA_B$ receptors expression were decreased in cerebral cortex of pilocarpine-induced epileptic rats (Mathew et al., 2012).

In addition to GABA and glutamate, many signal molecules in the brain such as acetylcholine, nitric oxide, cytokines and oxytocin also contribute to the neuropathology of seizures (Erfanparast et al., 2017; Wang et al., 2021; Boroujeni et al., 2022). Pharmacological findings have suggested the use of cannabinoids in the management of epilepsy (Arslan, et al., 2017; Cheung et al., 2019). For example, anticonvulsive effects of cannabinoid compounds in various animal models of epilepsy, such as PTZ model in mice (Gholizadeh et al., 2007) and penicillin model of epileptiform activity in rats

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(Arslan et al., 2019) have been reported. In addition, microinjection of anandamide (AEA), an endogenous cannabinoid and WIN55,212, a non-selective agonist of CB_1 receptor into the ventroposteromedial thalamic nucleus (VPM) reduced absence seizures in the WAG/Rij rats (Citraro et al., 2013). Experimental evidences have suggested potential roles for serotonin and related chemical compounds in epilepsy regulation (Sourbron & Lagae, 2022). For example, a selective 5-HT_{2C} antagonist, SB 242984, blocked the effects of lorcaserin and CP-809,101 (agonists of 5-HT_{2C} receptors) in absence seizures (Venzi et al., 2016). In addition, pre-treatment with SB269970, a selective antagonist of $5HT_7$ receptor, prevented epileptogenic effect of AS19, a selective agonist of 5-HT_7 receptor in pilocarpine-induced epilepsy (Yang et al., 2012).

Some brain functions are affected by interference between cannabinoid and serotonin systems. For example, pre-treatment with parachlorophenylalanine (PCPA, 5-HT-depleting agent) abolished the antidepressant-like effect induced by WIN55,212-2, an agonist of CB_1 receptor (Bambico et al., 2007). Moreover, intraperitoneal administration of CP55,940, a non-selective agonist of cannabinoid receptor, enhanced 5-HT_{2A} receptors expression in prefrontal cortex (Franklin & Carrasco, 2012). In addition, pre-treatment with WIN55,212-2 counteracted pilocarpine-induced 5-HT_{2C} receptors downregulation (Di Maio et al., 2019).

Although reducing effect following intracerebroventricular (ICV) injection of arachidonyl-2-chloroethylamide (ACEA, an agonist of CB_1 receptor) in penicillin model of epilepsy has been reported (Kozan et al., 2009), CB_1 and 5-HT_{1A} receptors involvement and interaction have not been directly investigated regarding the primary somatosensory cortex (S1). The S1 has potent roles in epilepsy regulation (Depaulis & Charpier, 2018), and contains high densities of CB_1 and 5-H_{1A} receptors (Mackie, 2005; Kropf et al., 2019). Therefore, this study was aimed to find out function as well as interaction of CB_1 and 5-HT_{1A} receptors by intra-S1 microinjection of ACEA (an agonist of CB_1 receptor), AM-251 (an antagonist of CB_1 receptor), 8-OH-DPAT (an agonist of 5-HT_{1A} receptor) and WAY-100635 (an antagonist of 5-HT_{1A} receptor), in epileptiform activity induced by the same site administration of penicillin. In addition to the well-known inhibitory effect on GABA receptors (Rossokhin et al., 2014), penicillin also employs other mechanisms such as P2X7 receptor - T-type calcium ion channel interaction, mitochondrial dysfunction, nitric oxide alteration and oxidative and inflammatory mechanisms to produce epileptiform activity (Arslan et al., 2019; Aygun & Bilginoglu, 2020; Wanleenuwat et al., 2020; Türel et al., 2022).

METHODS

Animals

Male Wistar rats (180–200 g) that were used in this study, were kept under standard conditions (22 ± 0.5°C; 12 h–12 h dark-light cycle; with lights turned on at 07:00 h) with unrestricted access to food and water. All experiments were conducted during the time between 9:00 h and 15:00 h. All experimental protocols were approved by Veterinary Ethics Committee of Urmia University Faculty of Veterinary Medicine (ethical code: IR-UU-AEC-3/31-19-7-2023).

Chemicals

Chemical including arachidonyl-2-chloroethylamide (ACEA, an agonist of CB_1 receptor), 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (AM-251, an antagonist of CB_1 receptor), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, an agonist of 5-HT_{1A} receptor) and N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide (WAY-100635, an antagonist of 5-HT_{1A} receptor) were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Penicillin G potassium was purchased from Jaber-Ebne-Hayyan Pharmaceutic. Co., Tehran, Iran. Urethane was purchased from Merck Co., Darmstadt, Germany. The drug solutions were freshly prepared.

Study protocol

The time line and administration protocol have been presented in Fig. 1. Animals were anesthetized and fixed in stereotaxic apparatus at 30 and 20 min before intra-S1 microinjection of penicillin. Alone and co-administration of antagonists (AM-251 and WAY-100635) and agonists (ACEA and 8-OH-DPAT) into the S1 were done 15 min after penicillin microinjection. The electrocorticographic (ECoG) activity was recorded from 10 min before penicillin administration and lasted up to 90 min after administration of drugs. Ten minutes after the end of the ECoG recording, the brains were taken out.

Experimental groups

Seventy-two rats were divided into 12 groups of six as follows:

Group 1 (Vehicle group) received intra-S1 microinjection of vehicle after the same site administration of penicillin.

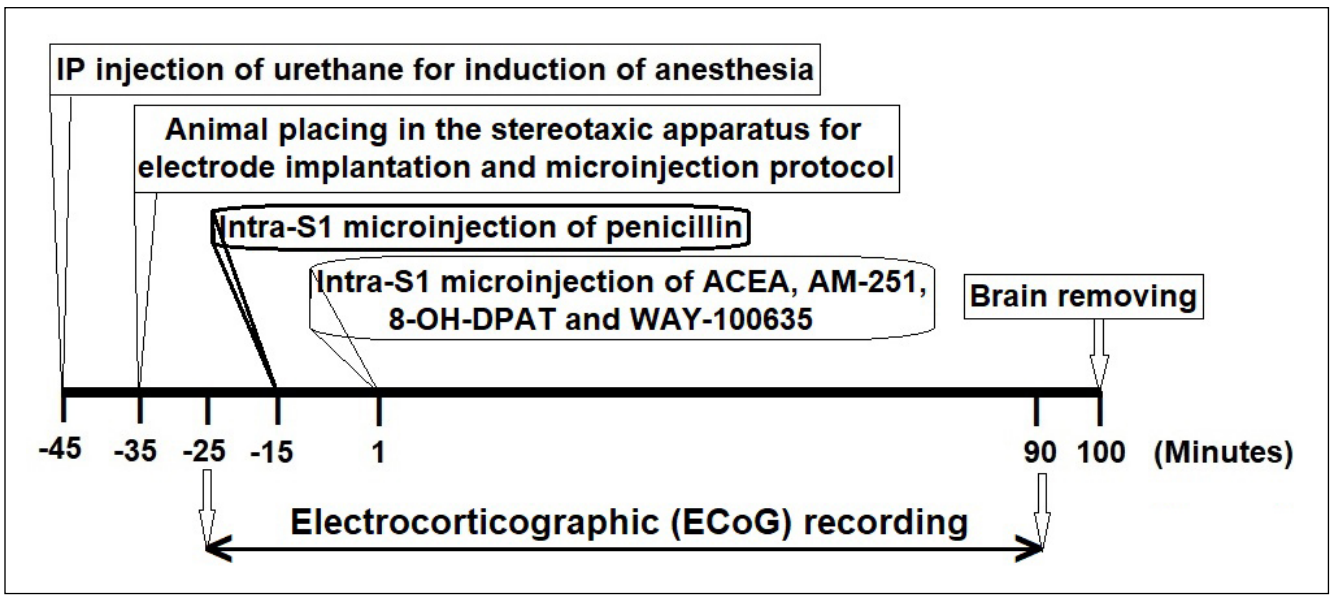

Fig. 1. Time line, microinjection protocol and electrocorticographic recording procedure used in the present study. Animals were anesthetized and placed in stereotaxic device at 35 and 25 min before intra-S1 microinjection of penicillin, respectively. Alone and co-administration of antagonists (AM-251 and WAY-100635) and agonists (ACEA and 8-OH-DPAT) into the S1 were done 15 min after penicillin microinjection. The ECoG activity was recorded from 10 min before penicillin administration and lasted up to 90 min after administration of drugs. Ten minutes after the end of the ECoG recording, the brains were removed.

Groups 2 (ACEA 10 group) and 3 (ACEA 50 group) were treated with intra-S1 microinjection of ACEA at doses of 10 and 50 ng, respectively, after the same site microinjection of penicillin.

Groups 4 (8-OH-DPAT 100 group) and 5 (8-OH-DPAT 500 group) received intra-S1 microinjection of 8-OH-DPAT at doses of 100 and 500 ng, respectively, after the same site penicillin administration.

Group 6 (ACEA 10+8-OH-DPAT 100 group) was treated with intra-S1 co-administration of low doses of ACEA (10 ng) plus 8-OH-DPAT (100 ng), after the same site microinjection of penicillin.

Group 7 (AM-251 50 group) received intra-S1 microinjection of AM-251 at a dose of 50 ng, after the same site penicillin microinjection.

Groups 8 (AM-251 50+ACEA 50 group) and 9 (AM-251 50+8-OH-DPAT 500 group) were treated with intra-S1 co-administration of 50 ng AM-251 plus 50 ng ACEA and 50 ng AM-251 plus 500 ng 8-OH-DPAT, respectively, after the same site microinjection of penicillin.

Group 10 (WAY-100635 500 group) was treated with intra-S1 microinjection of WAY-100635 at a dose of 500 ng, after intra-S1 administration of penicillin.

Groups 11 (WAY-100635 500+8-OH-DPAT 500 group) and 12 (WAY-100635 500+ACEA 50 group) were treated with intra-S1 administration of 500 ng WAY-100635 plus 500 ng 8-OH-DPAT and 500 ng WAY-100635 plus 50 ng ACEA, after the same site penicillin administration.

In the present study, the used doses of drugs were in accordance with other investigations (Dos Santos et al., 2008; Nasehi et al., 2009) and our preliminary studies.

Intra-S1 microinjection

After placing of anesthetized animal (1.2 mg/kg, urethane) in a stereotaxic device (Stoelting, Wood Lane, IL, USA), one hole with a diameter of 1 mm was made in the skull bone with the following coordinates (Paxinos & Watson, 2007): 2 mm posterior to the bregma and 2.6 mm lateral to midline. For microinjection into the S1, the tip of a 5 µL Hamilton syringe was placed at a depth of 2.4 mm from the surface of the skull bone. The volumes of test drugs and penicillin for microinjection into the S1 were 0.5 and 1 µL, respectively.

Epileptic focus induction and ECoG recording

The epileptic focus was induced by intra-S1 microinjection of 200 IU/1 µL penicillin G potassium over a 120-s time period (Tamaddonfard et al., 2012). The ECoG activity was recorded using DataLab 2000 system, Lafayette Instrument, USA. Three electrodes with a length of 5 mm and a diameter of 0.5 mm were used to record the ECoG. The frontal and parietal electrodes were implanted 2 mm anterior and 4 mm poste-

rior to the bregma, respectively, with equal distances of 2.5 mm from the midline (Paxinos & Watson, 2007). A reference electrode was inserted on body skin. The electrode wires were attached to a general-purpose interface bed (GPI bed, model 70701, Lafayette Instrument Co., USA) through a biopotential amplifier (model 70702, Lafayette Instrument Co., USA) and finally to a computer. To observe the basal waves, ECoG recording started 15 min before and continued until 90 min after penicillin microinjection. The parameters including frequency and amplitude of spike waves in 15-min intervals were calculated offline.

Verification of intra-S1 microinjected site

Ten minutes after completing the recording of the ECoG, 1 µL of methylene blue was microinjected into the S1. Immediately after induction of deep anesthesia with intracardiac injection of 0.5 ml of 10 % ketamine, the animals were decapitated and the brains were brought out and dropped in a formalin (10 %) solution. Four days later, the cortical site (S1) of administration was controlled. Validation of the microinjection site in the S1 brain section (A) and plate taken from the atlas of Paxinos & Watson, 2007 (B) have been shown in Fig. 2.

Statistical analysis

Graph Pad Prism 8.2.1 (GraphPad Software Inc., San Diego, CA, USA) used to analyze obtained data. Time-point related results were analyzed using two-way repeated measures ANOVA (analysis of variance) and then with Bonferroni's test. The results obtained from areas under the curves (AUC) were analyzed using one-way ANOVA followed by Tukey's test. The AUC was calculated by trapezoid method (Cardoso et al., 2011). All data have been presented as mean ± SEM. The statistical significance of *P* less than 0.05 was considered for all results.

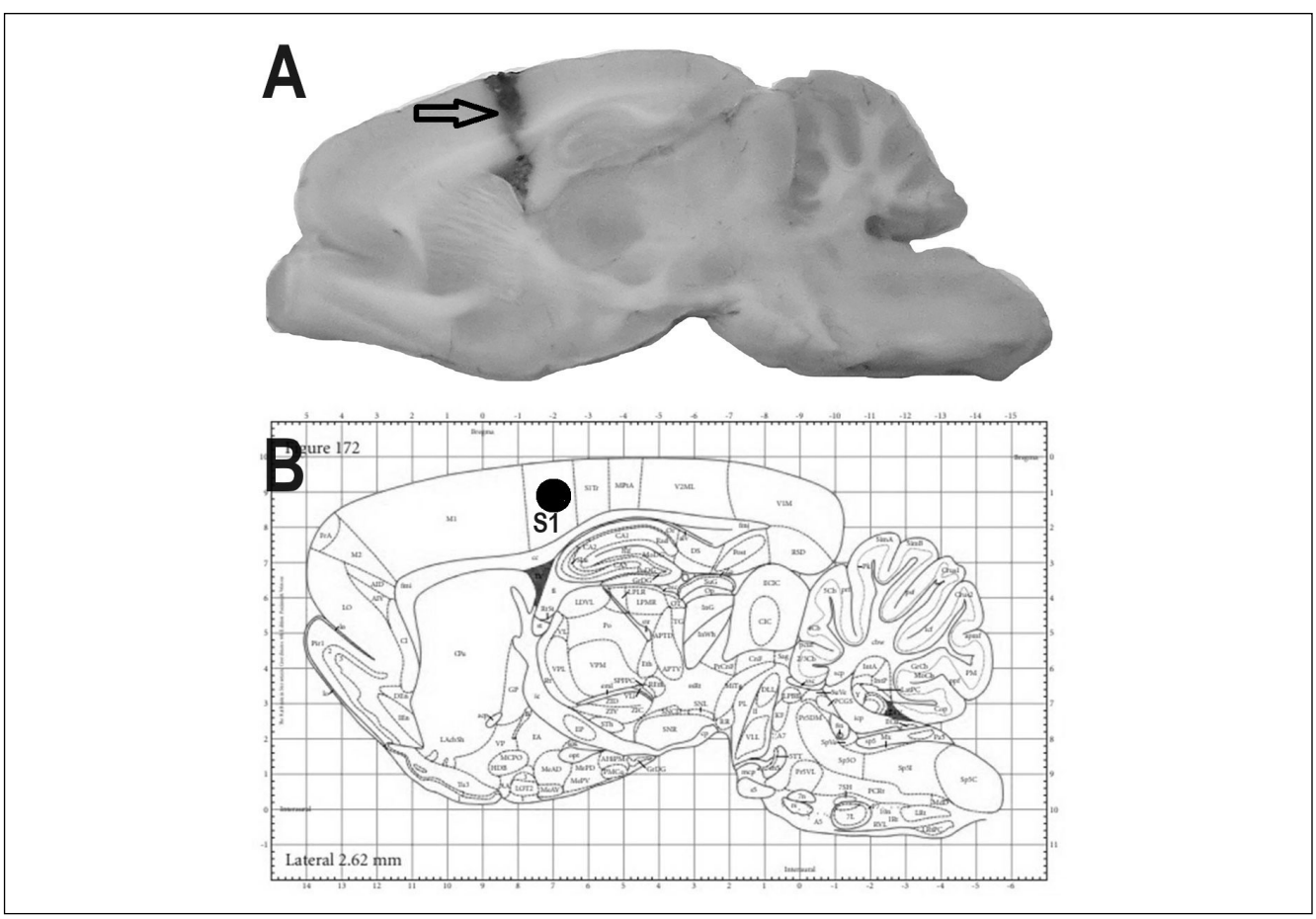

Fig. 2. Longituidinal section (A) and schematic atlas plate (B) of the S1 of the brain showing the position of microinjection site. Hollow white arrow (A) and black circle (B) indicate intra-S1 microinjection site. S1: Primary somatosensory cortex. The plates adapted from Paxinos and Watson stereotaxic atlas of the brain of rats (Paxinos & Watson, 2007).

RESULTS

ECoG recording alterations obtained from the present study

Fig. 3 shows the ECoG recording alterations obtained from the present study. Penicillin (200 IU) induced spike waves with high frequency and amplitude (Fig. 3A). ACEA (50 ng, Fig. 3C), 8-OH-DPAT (500 ng, Fig. 3E) and 10 ng ACEA plus 100 ng 8-OH-DPAT (Fig. 3F) decreased spike waves frequency and amplitude. ACEA (10 ng, Fig. 3B) and 8-OH-DPAT (100 ng, Fig. 3D) had no effects. AM-251 alone (50 ng, Fig. 3G) increased spike waves frequency, and prior microinjection of AM-251 inhibited the effects of ACEA (50 ng, Fig. 3H) and 8-OH-DPAT (500 ng, Fig. 3I). WAY-100635 alone (500 ng, Fig. 3J) increased spike waves frequency, and reversed the reducing effects induced by 8-OH-DPAT (500 ng, Fig. 3K) and ACEA (50 ng, Fig. 3L). A typical ECoG during the basal brain activity of an anesthetized rat can be seen in Fig. 3M. The first spike wave has been indicated by the hollow white arrow in Fig. 3N.

Effects of separate and combined intra-S1 microinjections of ACEA, AM-251, 8-OH-DPAT and WAY-100635 on spike waves frequency

Fig. 4 shows the effects of separate and combined intra-S1 microinjections of ACEA, AM-251, 8-OH-DPAT

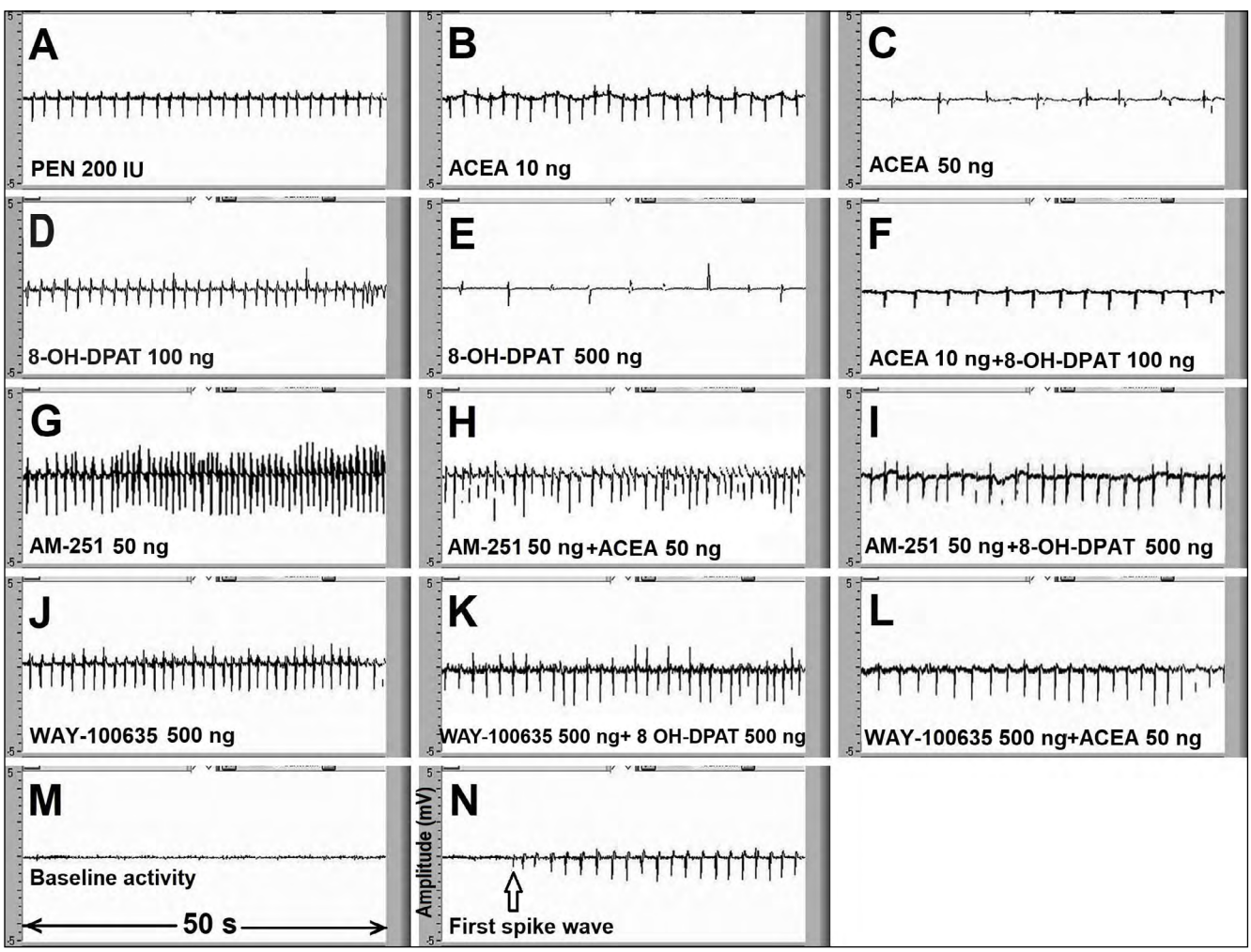

Fig. 3. The ECoG recordings samples obtained from right primary somatosensory cortex (S1). (A) epileptiform activity induced by microinjection of penicillin into the S1. (B) and (C) shows the effects of intra-S1 microinjection of 10 and 50 ng of ACEA, respectively. (D) and (E) shows the effects of intra-S1 microinjection of 100 and 500 ng 8-OH-DPAT, respectively. (F) shows co-administration of ACEA (10 ng) and 8-OH-DPAT (100 ng). The effect of intra-S1 microinjected AM-251 (50 ng) is shown in (G). The effects induced by co-administration of AM-251 (50 ng) with ACEA (50 ng) and 8-OH-DPAT (500 ng) are shown in (H) and (I), respectively. The effect of intra-S1 microinjection of WAY-100635 (500 ng) is shown in (J). The effects of co-administration of WAY-100635 (500 ng) with 8-OH-DPAT (500 ng) and ACEA (50 ng) are shown in (K) and (L), respectively. (M) shows the baseline activity. (N) shows the first spike wave (white arrow).

and WAY-100635 on spike waves frequency. Spike waves frequency induced by penicillin was 30.14±1.45 No/min. The ACEA (50 ng) and 8-OH-DPAT (500 ng) reduced all 15-min time points of spike waves frequency (Fig. 4A). In addition, ACEA (10 ng) plus 8-OH-DPAT (100 ng) decreased the second-fourth 15-min time points of spike waves frequency (Fig. 4A). The AM-251 (50 ng) alone increased first-fifth 15-min time points of spike waves frequency (Fig. 4B) and in co-microinjection treatment, it reversed the decreased spike waves frequency induced by ACEA (50 ng) and 8-OH-DPAT (500 ng) (Fig. 4B). The WAY-100635 alone was without effect, whereas inhibited the reducing effects of 500 ng 8-OH-DPAT and 50 ng ACEA on spike waves frequency (Fig. 4C). Corresponding AUC confirmed the significant effects induced by the above-mentioned treatments on spike waves frequency (Fig. 4D).

Effects of separate and combined intra-S1 microinjections of ACEA, AM-251, 8-OH-DPAT and WAY-100635 on amplitude of spike waves

Fig. 5 shows the effects of separate and combined intra-S1 microinjections of ACEA, AM-251, 8-OH-DPAT and WAY-100635 on spike waves amplitude. The aver-

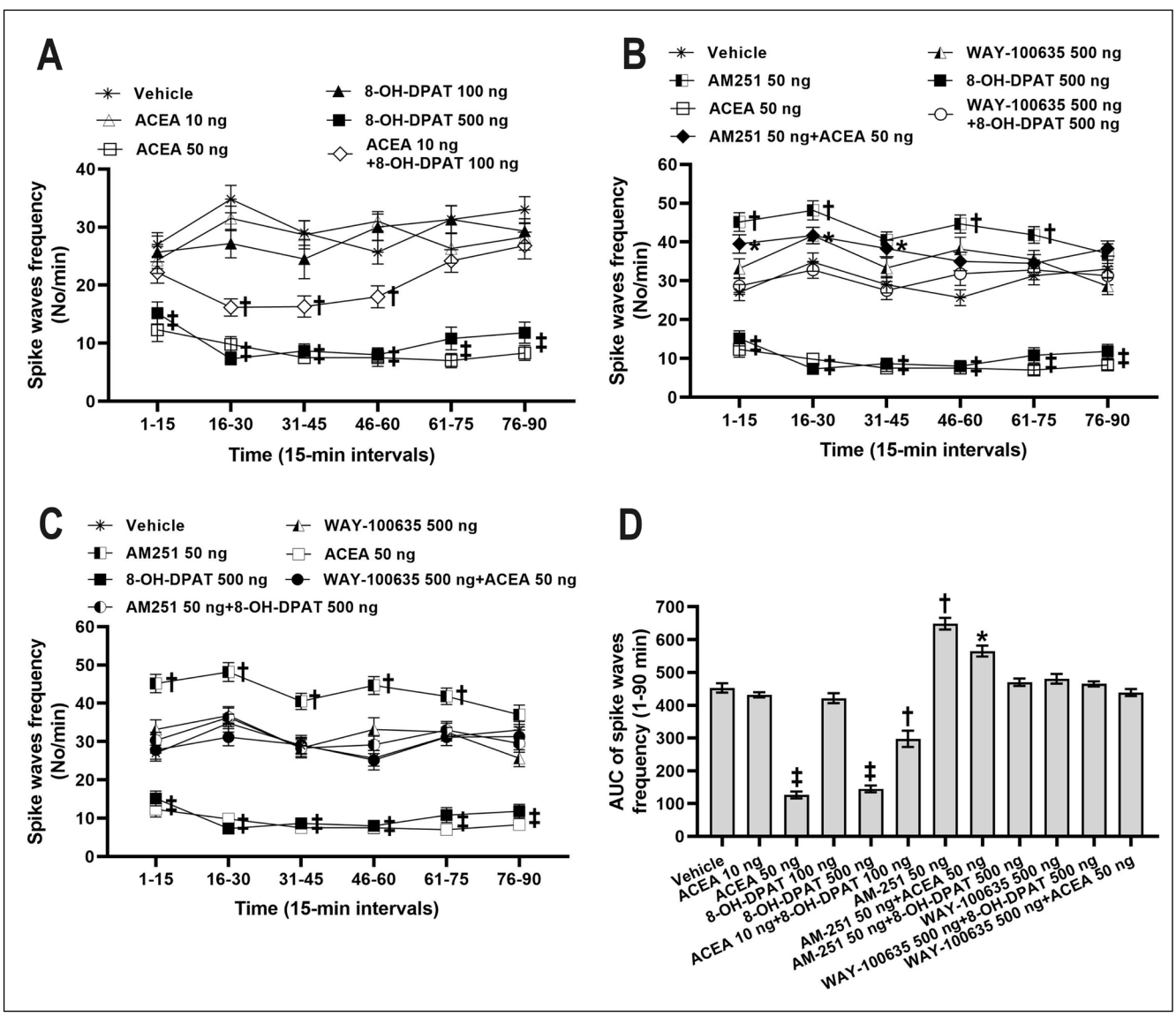

Fig. 4. The effects of alone and co-microinjections of ACEA, 8-OH-DPAT (A), alone and co-microinjection of AM-251 with ACEA and 8-OH-DPAT (B) and alone and co-microinjection of WAY-100635 with 8-OH-DPAT and ACEA (C) at 15-min time intervals and corresponding AUC (D) on spike waves frequency. Alone and co-microinjections of test drugs into the S1 were performed 15 min after penicillin microinjection into the same site. The mean ± SEM was applied for expressing values (n=6). * $P<0.05$, † $P<0.01$ and ‡ $P<0.001$ *vs.* vehicle.

age amplitude of penicillin-induced spike waves was 1.27 mV. The ACEA (50 ng) and 8-OH-DPAT (500 ng) reduced all 15-min time points of spike waves amplitude (Fig. 5A). In addition, ACEA (10 ng) plus 8-OH-DPAT (100 ng) decreased the second-fourth 15-min time points of spike waves amplitude (Fig. 5A). The AM-251 (50 ng) alone was without effect, whereas in co-microinjection treatment, it reversed the reducing effects of 50 ng ACEA and 500 ng 8-OH-DPAT on waves amplitude (Fig. 5B). The WAY-100635 alone had no effects, whereas the reducing effects of 500 ng 8-OH-DPAT and 50 ng ACEA on spike waves amplitude were inhibited by co-microinjection of WAY-100635 (Fig. 5C). The above-mentioned treatment effects on spike waves amplitude were supported by corresponding AUC (Fig. 5D).

DISCUSSION

In this study, spike waves with frequency of 28-34 spike/min and amplitude of 1.2-1.4 mV were determined after intra-S1 microinjection of penicillin. Penicillin microinjection into different areas of the brain including motor cortex, sensory cortex, hippocampus is widely used as a chemical model to investigate electrophysiological mechanisms and behavioral responses

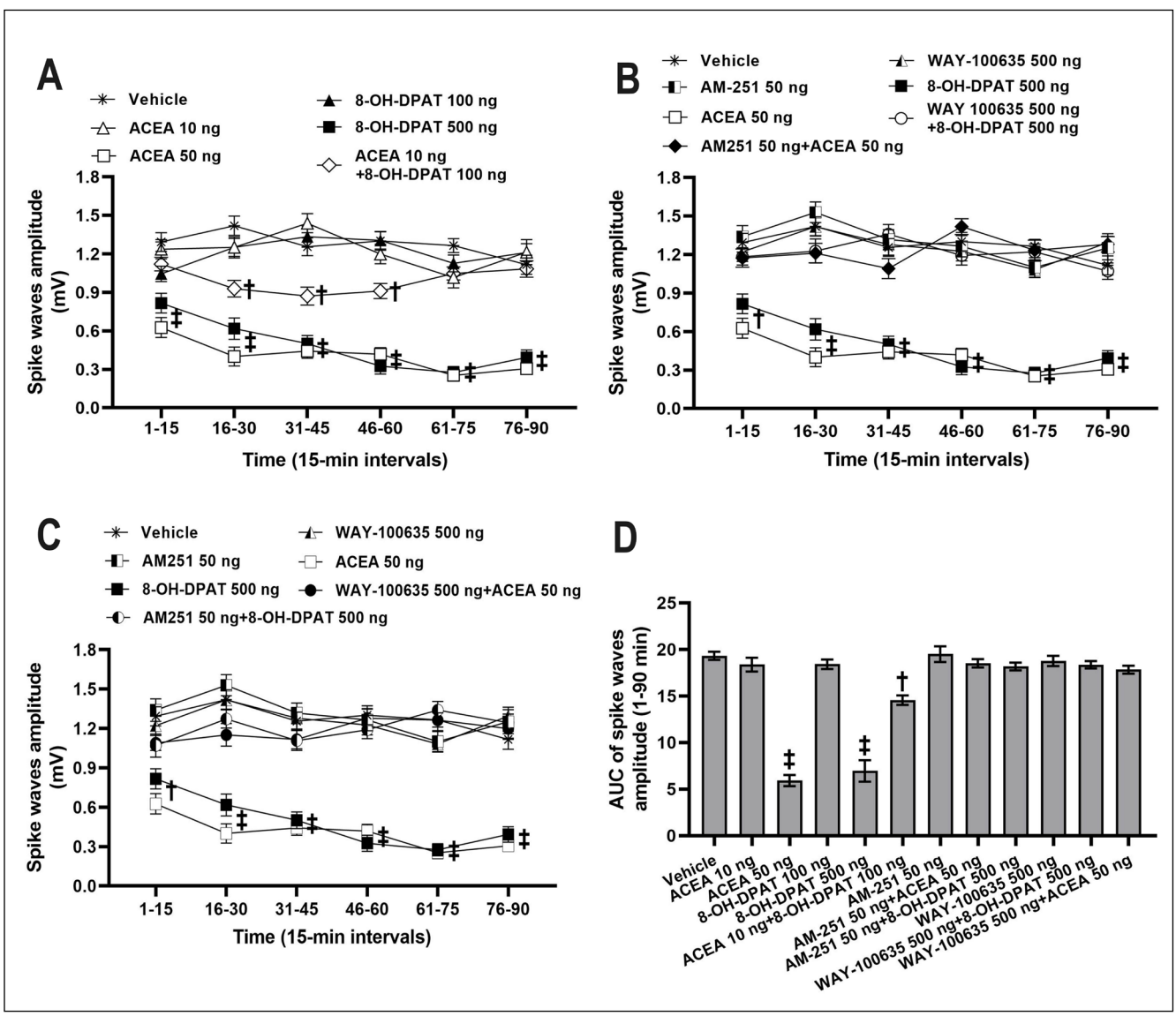

Fig. 5. The effects of alone and co-microinjections of ACEA, 8-OH-DPAT (A), alone and co-microinjection of AM-251 with ACEA and 8-OH-DPAT (B) and alone and co-microinjection of WAY-100635 with 8-OH-DPAT and ACEA (C) at 15-min time intervals and corresponding AUC (D) on spike waves amplitude. Alone and co-microinjections of test drugs into the S1 were performed 15 min after penicillin microinjection into the same site. The mean ± SEM was applied for expressing values (n=6). † P<0.01 and ‡ P<0.001 *vs.* vehicle.

to antiepileptic and epileptogenic drugs (Tamaddonfard et al., 2012; Erfanparast & Tamaddonfard, 2015; Tasdemir et al., 2018; Taskiran et al., 2019; Musuroglu Keloglan et al., 2023). Referring to the above-mentioned studies, 25-32 spike/min with amplitude of 1.1-1.5 mV have been completely evident (Tamaddonfard et al., 2012; Erfanparast & Tamaddonfard, 2015; Tasdemir et al., 2018; Taskiran et al., 2019; Musuroglu Keloglan et al., 2023). Therefore, the results of the present study support the aforementioned findings.

The ECoG recordings of the present study showed that ACEA decreased epileptic spike waves number and amplitude at the level of the S1, and this effect was inhibited by AM-251. Moreover, AM-251 alone increased spike waves frequency. Therefore, CB_1 receptors involvement in penicillin epilepsy was cleared by the results of the present study. To this date, an explanation indicating the direct role of the cannabinoid system in the S1 processing of electroencephalographic (EEG) changes caused by penicillin microinjection into the same region has not been provided. However, ICV injection of AM-251 and ACEA alone increased and decreased epileptiform activity induced by sensorimotor microinjection of penicillin, respectively, and prior administration of AM-251 inhibited the reducing effect of ACEA (Kozan et al., 2009). Similar proconvulsive and anticonvulsive effects of ICV-injected AM-251 and ACEA, respectively, have been reported in PTZ-induced epileptic rats which determined by EEG and behavioral outcomes (Al-Kaleel et al., 2023). Immunohistochemical findings have revealed the distribution of CB_1 receptors from cerebral cortex areas to the dorsal horn of the spinal cord in rats (Tsou et al., 1998). In comparison with other brain structures, the CB_1 receptors with moderate density are distributed in all cortical layers of the rat brain (Bodor et al., 2005). Electrophysiological data have suggested that CB_1 receptors are involved in neuronal excitability. For example, SR141716A, a CB_1 receptor antagonist, antagonized the suppressive effect of WIN55212-2 (a CB_1 receptor agonist) on spike activity in basolateral amygdala neurons evoked by medial prefrontal cortex electrical stimulation (Pistis et al., 2004). In maximal dentate activation and pilocarpine-induced acute seizures models of temporal lobe epilepsy, WIN55212-2 reduced electrically-induced epileptiform discharge, decreased pilocarpine-induced acute seizures and increased latency of acute convulsions (Carletti et al., 2015). Considering the above findings, the results of the present study suggest S1-CB_1 receptor contribution in penicillin-induced epileptiform activity.

Present study ECoG results indicated that 8-OH-DPAT alleviated penicillin-induced epileptiform activity, and this effect was prevented by co-administration of WAY-100635. The results presented here, express the contribution of 5-HT_{1A} receptors in penicillin-induced epilepsy. The 5-HT_{1A} receptors are widely distributed in all areas of the rat brain including cerebral cortex, hippocampus, brain stem nuclei and spinal cord (Pompeiano et al., 1992), and involved in modulation of the brain functions such as anxiety, depression and epilepsy (Popova et al., 2013; Sourbron & Lagae, 2022). Although there are no reports showing 5-HT_{1A} receptor involvement in penicillin-induced epileptiform activity, WAY-100635 blocked the anti-seizure effects of 8-OH-DPAT and indorinate (a 5-HT1A receptor agonist) in kainic acid-induced limbic seizures and amygdala kindling epilepsy (López-Meraz et al., 2005). In the PTZ model of epilepsy, 8-OH-DPAT, increased first myoclonic jerk (FMJ) time and decreased spike-wave discharge (SWD) per minute (Sahin et al., 2019). To confirm the inhibitory effect of activation of 5-HT_{1A} receptors on electrical activity, it should be noted that 5-HT and 8-OH-DPAT produced inhibitory effects on spontaneous unit discharge in the S1. It has been reported that application of serotonin and 8-OH-DPAT into the medium containing slices of the CA_1 area of the hippocampus causes reduction of spontaneous epileptiform activity (Tokarski, et al., 2002).

The results of the present study showed that intra-S1 co-microinjection of low (ineffective) doses of ACEA (10 ng) and 8-OH-DPAT (100 ng) produced antiepileptic activity. In addition, ACEA (50 ng)-induced suppression of epileptic spikes was inhibited by WAY-100635 (500 ng). Moreover, AM-251 (50 ng) prevented the attenuating effect of 500 ng 8-OH-DPAT on penicillin-induced epileptiform activity. These results expressed a cross interaction between CB_1 and 5-HT_{1A} receptors at the S1 level in modulation of penicillin-induced epileptiform activity. Functional interaction between CB_1 and 5-HT_{1A} receptors has been reported in a number of brain functions. For example, alone and a co-treatment with cannabidiol (CBD) and 8-OH-DPAT decreased motor activity, and WAY-100635 prevented the effects of CBD and 8-OH-DPAT (Espejo-Porras et al., 2013). Using forced-swimming test, reduction of immobility time induced by microinjection of CBD and 8-OH-DPAT into the ventral medial prefrontal cortex (vmPFC) blocked by the same site WAY-100635 pre-administration (Sartim et al., 2016). In the G protein-coupled receptor system (GPCR), both CBD and 5-HT decreased cAMP concentration at similar apparent levels of 5-HT_{1A} receptor occupancy suggesting agonistic action of CBD at the human 5-HT_{1A} receptor (Russo et al., 2005). Based on afore-mentioned findings, it seems that the present study may represent the first report on the functional interaction between CB_1 and 5-HT_{1A} receptors in the processing of penicillin-induced epileptiform activity.

The results presented here showed that ACEA and 8-OH-DPAT attenuated penicillin G potassium-induced epileptiform activity. Penicillin G, as a protype β-lactam antibiotic, suppresses GABAergic neurotransmission in the central nervous system by blocking a voltage-dependent open channel of $GABA_A$ receptor (Rossokhin et al., 2014). In the CB_1 receptor knockout mice, reduction of $GABA_A$ receptor densities in the hippocampus, thalamus and cerebral cortex have been reported (Urigüen et al., 2011). Microinjection of WIN55212-2 into the central nucleus of the amygdala (CeA) impaired memory retrieval and this effect was potentiated by co-microinjection of muscimol (a $GABA_A$ receptor agonist) and prevented by prior intra-CeA administration of a $GABA_A$ receptor antagonist, bicuculline (Hasanein & Sharifi, 2015). It has been reported that $5\text{-}HT_{1A}$ receptor null mice exhibit anxiety-related behaviors, do not respond to benzodiazepine (BZ), have reduced BZ binding, and have decreased expression of the major $GABA_A$ receptor subunits $alpha_1$ and $alpha_2$ (Bailey & Toth, 2004). In addition, in the dorsal lateral geniculate nucleus (dLGN), phasic inhibitory post-synaptic currents induced by synaptic $GABA_A$ receptors were enhanced by 5-HT-, and 8-OH-DPAT-activated $5\text{-}HT_{1A}$ receptor (Crunelli & Giovanni, 2015). Therefore, the antiepileptic effects caused by the activation of CB_1 and $5\text{-}HT_{1A}$ receptors observed in the present study can be explained by the interaction among the cannabinoid, serotonin and GABA systems.

CONCLUSION

The results presented here showed that intra-S1 microinjection of penicillin produced spike waves characterized by approximately 30 spike per minute with 1.4 mV amplitude. Alone and co-microinjection of ACEA and 8-OH-DPAT reduced penicillin-induced epileptiform activity indicating the involvement of CB_1 and $5\text{-}HT_{1A}$ receptors. Co-microinjection of AM-251 and WAY-100365 with ACEA and 8-OH-DPAT prevented the latter chemical compounds suppressing effects suggesting CB_1 and $5\text{-}HT_{1A}$ mediated mechanisms. Because penicillin G, itself is an antagonist of $GABA_A$ receptor, the produced antiepileptic effects might be extended to contribution among the cannabinoid, serotonin and GABA systems.

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REFERENCES

Akyuz, E., Polat, A. K., Eroglu, E., Kullu, I., Angelopoulou, E., & Paudel, Y. N. (2021). Revisiting the role of neurotransmitters in epilepsy: An updated review. *Life sciences*, 265, 118826. https://doi.org/10.1016/j.lfs.2020.118826

Al-Kaleel, A., Aygun, H., Al-Gailani, L., Kabak, Y., Inal, S., Ayyildiz, M., Him, A., & Agar, E. (2023). The electrophysiological and behavioral evaluation of the peptide hemopressin and cannabinoid CB1 receptor agonist and antagonist in pentylenetetrazol model of epilepsy in rats. *Pflugers Archiv* 475(6), 719–730. https://doi.org/10.1007/s00424-023-02814-y

Arslan, G., Alici, S. K., Ayyildiz, M., & Agar, E. (2017). Interaction between urethane and cannabinoid CB1 receptor agonist and antagonist in penicillin-induced epileptiform activity. *Acta neurobiologiae experimentalis*, 77(2), 128–136. https://doi.org/10.21307/ane-2017-045

Arslan, G., Avci, B., Kocacan, S. E., Rzayev, E., Ayyildiz, M., & Agar, E. (2019). The interaction between P2X7Rs and T-type calcium ion channels in penicillin-induced epileptiform activity. *Neuropharmacology*, *149*, 1–12. https://doi.org/10.1016/j.neuropharm.2019.01.027

Aygun, H., & Bilginoglu, A. (2020). Effect of tadalafil and nitric oxide agonist sodium nitroprusside on penicillin-induced epileptiform activity. *Neurological research*, *42*(1), 39–46. https://doi.org/10.1080/01616412.2019.1703166

Bailey, S. J., & Toth, M. (2004). Variability in the benzodiazepine response of serotonin 5-HT1A receptor null mice displaying anxiety-like phenotype: evidence for genetic modifiers in the 5-HT-mediated regulation of GABA(A) receptors. *The Journal of neuroscience*, *24*(28), 6343–6351. https://doi.org/10.1523/JNEUROSCI.0563-04.2004

Bambico, F. R., Katz, N., Debonnel, G., & Gobbi, G. (2007). Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *The Journal of neuroscience*, *27*(43), 11700–11711. https://doi.org/10.1523/JNEUROSCI.1636-07.2007

Bodor, A. L., Katona, I., Nyíri, G., Mackie, K., Ledent, C., Hájos, N., & Freund, T. F. (2005). Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *The Journal of neuroscience*, *25*(29), 6845–6856. https://doi.org/10.1523/JNEUROSCI.0442-05.2005

Boroujeni, S. S., Solaimanian, S., Jafari, R. M., Sabzevari, O., Shafaroodi, H., Maleki, A., Mohammadi, P., Karimi, E., & Dehpour, A. R. (2022). Opioidergic and nitrergic systems mediate the anticonvulsant effect of mefloquine and chloroquine on seizures induced by pentylenetetrazol and maximal electroshock in mice. *Acta neurobiologiae experimentalis*, *82*(2), 157–169. https://doi.org/10.55782/ane-2022-014

Cardoso, F. C., Sears, W., LeBlanc, S. J., & Drackley, J. K. (2011). Technical note: comparison of 3 methods for analyzing areas under the curve for glucose and nonesterified fatty acids concentrations following epinephrine challenge in dairy cows. *Journal of dairy science*, *94*(12), 6111–6115. https://doi.org/10.3168/jds.2011-4627

Carletti, F., Gambino, G., Rizzo, V., Ferraro, G., & Sardo, P. (2015). Cannabinoid and nitric oxide signaling interplay in the modulation of hippocampal hyperexcitability: Study on electrophysiological and behavioral models of temporal lobe epilepsy in the rat. *Neuroscience*, *303*, 149–159. https://doi.org/10.1016/j.neuroscience.2015.06.047

Cheung, K. A. K., Peiris, H., Wallace, G., Holland, O. J., & Mitchell, M. D. (2019). The Interplay between the Endocannabinoid System, Epilepsy and Cannabinoids. *International journal of molecular sciences*, *20*(23), 6079. https://doi.org/10.3390/ijms20236079

Citraro, R., Russo, E., Ngomba, R. T., Nicoletti, F., Scicchitano, F., Whalley, B. J., Calignano, A., & De Sarro, G. (2013). CB1 agonists, locally applied to the cortico-thalamic circuit of rats with genetic absence epilepsy, reduce epileptic manifestations. *Epilepsy research*, *106*(1-2), 74–82. https://doi.org/10.1016/j.eplepsyres.2013.06.004

Cremer, C. M., Palomero-Gallagher, N., Bidmon, H. J., Schleicher, A., Speckmann, E. J., & Zilles, K. (2009). Pentylenetetrazole-induced seizures affect binding site densities for GABA, glutamate and adenosine

receptors in the rat brain. *Neuroscience*, *163*(1), 490–499. https://doi.org/10.1016/j.neuroscience.2009.03.068

Crunelli, V., & Di Giovanni, G. (2015). Differential Control by 5-HT and 5-HT1A, 2A, 2C Receptors of Phasic and Tonic GABAA Inhibition in the Visual Thalamus. *CNS neuroscience & therapeutics*, *21*(12), 967–970. https://doi.org/10.1111/cns.12480

Depaulis, A., & Charpier, S. (2018). Pathophysiology of absence epilepsy: Insights from genetic models. *Neuroscience letters*, *667*, 53–65. https://doi.org/10.1016/j.neulet.2017.02.035

Di Maio, R., Colangeli, R., & Di Giovanni, G. (2019). WIN 55,212-2 Reverted Pilocarpine-Induced Status Epilepticus Early Changes of the Interaction among 5-HT_{2C}/NMDA/CB_1 Receptors in the Rat Hippocampus. *ACS chemical neuroscience*, *10*(7), 3296–3306. https://doi.org/10.1021/acschemneuro.9b00080

Dos Santos, L., de Andrade, T. G., & Zangrossi Junior, H. (2008). 5-HT1A receptors in the dorsal hippocampus mediate the anxiogenic effect induced by the stimulation of 5-HT neurons in the median raphe nucleus. *European neuropsychopharmacology*, *18*(4), 286–294. https://doi.org/10.1016/j.euroneuro.2007.07.007

Erfanparast, A., Tamaddonfard, E., & Henareh-Chareh, F. (2017). Intra-hippocampal microinjection of oxytocin produced antiepileptic effect on the pentylenetetrazol-induced epilepsy in rats. *Pharmacological reports : PR*, *69*(4), 757–763. https://doi.org/10.1016/j.pharep.2017.03.003

Erfanparast, A., & Tamaddonfard, E. (2015). Effects of intracortical microinjection of vitamin B12 on penicillin-induced epileptiform activity in rats. *Acta neurobiologiae experimentalis*, *75*(2), 200–207. https://doi.org/10.55782/ane-2015-2028

Espejo-Porras, F., Fernández-Ruiz, J., Pertwee, R. G., Mechoulam, R., & García, C. (2013). Motor effects of the non-psychotropic phytocannabinoid cannabidiol that are mediated by 5-HT1A receptors. *Neuropharmacology*, *75*, 155–163. https://doi.org/10.1016/j.neuropharm.2013.07.024

Franklin, J. M., & Carrasco, G. A. (2012). Cannabinoid-induced enhanced interaction and protein levels of serotonin 5-HT(2A) and dopamine D-receptors in rat prefrontal cortex. *Journal of psychopharmacology*, *26*(10), 1333–1347. https://doi.org/10.1177/0269881112450786

Gholizadeh, S., Shafaroodi, H., Ghasemi, M., Bahremand, A., Sharifzadeh, M., & Dehpour, A. R. (2007). Ultra-low dose cannabinoid antagonist AM251 enhances cannabinoid anticonvulsant effects in the pentylenetetrazole-induced seizure in mice. *Neuropharmacology*, *53*(6), 763–770. https://doi.org/10.1016/j.neuropharm.2007.08.005

Goodman, A. M., & Szaflarski, J. P. (2021). Recent Advances in Neuroimaging of Epilepsy. *Neurotherapeutics*, *18*(2), 811–826. https://doi.org/10.1007/s13311-021-01049-y

Hasanein, P., & Sharifi, M. (2015). GABA(A) receptors in the central amygdala are involved in memory retention deficits induced by cannabinoids in rats. *Pharmacology, biochemistry, and behavior*, *138*, 26–31. https://doi.org/10.1016/j.pbb.2015.09.010

Kozan, R., Ayyildiz, M., & Agar, E. (2009). The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. *Epilepsia*, *50*(7), 1760–1767. https://doi.org/10.1111/j.1528-1167.2009.02098.x

Kropf, E., Syan, S. K., Minuzzi, L., & Frey, B. N. (2019). From anatomy to function: the role of the somatosensory cortex in emotional regulation. *Revista brasileira de psiquiatria*, *41*(3), 261–269. https://doi.org/10.1590/1516-4446-2018-0183

López-Meraz, M. L., González-Trujano, M. E., Neri-Bazán, L., Hong, E., & Rocha, L. L. (2005). 5-HT1A receptor agonists modify epileptic seizures in three experimental models in rats. *Neuropharmacology*, *49*(3), 367–375. https://doi.org/10.1016/j.neuropharm.2005.03.020

Mackie K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handbook of experimental pharmacology*, (168), 299–325. https://doi.org/10.1007/3-540-26573-2_10

Mathew, J., Balakrishnan, S., Antony, S., Abraham, P. M., & Paulose, C. S. (2012). Decreased GABA receptor in the cerebral cortex of epileptic rats: effect of Bacopa monnieri and Bacoside-A. *Journal of biomedical science*, *19*(1), 25. https://doi.org/10.1186/1423-0127-19-25

Musuroglu Keloglan, S., Aycik, F. B., Kocacan, S. E., Yazgan, B., Ayyildiz, M., & Agar, E. (2023). Nesfatin1 exerts anticonvulsant effect by reducing oxidative stress in experimental epilepsy model. *Acta neurobiologiae experimentalis*, *83*(3), 227–235. https://doi.org/10.55782/ane-2023-2419

Nasehi, M., Sahebgharani, M., Haeri-Rohani, A., & Zarrindast, M. R. (2009). Effects of cannabinoids infused into the dorsal hippocampus upon memory formation in 3-days apomorphine-treated rats. *Neurobiology of learning and memory*, *92*(3), 391–399. https://doi.org/10.1016/j.nlm.2009.05.

Paxinos, G., Watson, C. (2007). The rat brain in stereotaxic coordinates. Elsevier Science &Technology.

Pistis, M., Perra, S., Pillolla, G., Melis, M., Gessa, G. L., & Muntoni, A. L. (2004). Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. *Neuropharmacology*, *46*(1), 115–125. https://doi.org/10.1016/j.neuropharm.2003.08.003

Pompeiano, M., Palacios, J. M., & Mengod, G. (1992). Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. *The Journal of neuroscience, 12*(2), 440–453. https://doi.org/10.1523/JNEUROSCI.12-02-00440.1992

Popova, N. K., & Naumenko, V. S. (2013). 5-HT1A receptor as a key player in the brain 5-HT system. *Reviews in the neurosciences*, *24*(2), 191–204. https://doi.org/10.1515/revneuro-2012-0082

Rossokhin, A. V., Sharonova, I. N., Bukanova, J. V., Kolbaev, S. N., & Skrebitsky, V. G. (2014). Block of GABA(A) receptor ion channel by penicillin: electrophysiological and modeling insights toward the mechanism. *Molecular and cellular neurosciences*, *63*, 72–82. https://doi.org/10.1016/j.mcn.2014.10.001

Russo, E. B., Burnett, A., Hall, B., & Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochemical research*, *30*(8), 1037–1043. https://doi.org/10.1007/s11064-005-6978-1

Şahin, B., Özdemir, E., Taşkiran, A.S., Gümüş, E., Ergül, M., (2019). Investigation of anti-epileptic mechanisms of 5HT1A receptor with pentylenetetrazole induced epilepsy model in rats. *Cumhuriyet Med J, 41*(3), 490–499.

Sartim, A. G., Guimarães, F. S., & Joca, S. R. (2016). Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex-Possible involvement of 5-HT1A and CB1 receptors. *Behavioural brain research*, *303*, 218–227. https://doi.org/10.1016/j.bbr.2016.01.033

Sarlo, G. L., & Holton, K. F. (2021). Brain concentrations of glutamate and GABA in human epilepsy: A review. *Seizure*, *91*, 213–227. https://doi.org/10.1016/j.seizure.2021.06.028

Sourbron, J., & Lagae, L. (2022). Serotonin receptors in epilepsy: Novel treatment targets? *Epilepsia open*, *7*(2), 231–246. https://doi.org/10.1002/epi4.12580

Tamaddonfard, E., Hamzeh Gooshchi, N., & Seiednejad-Yamchi, S. (2012). Central effect of crocin on penicillin-induced epileptiform activity in rats. *Pharmacological reports : PR*, *64*(1), 94–101. https://doi.org/10.1016/s1734-1140(12)70735-1

Taskiran, M., Tasdemir, A., Ayyildiz, N., Ayyildiz, M., & Agar, E. (2019). The effect of serotonin on penicillin-induced epileptiform activity. *The International journal of neuroscience*, *129*(7), 687–697. https://doi.org/10.1080/00207454.2018.1557166

Tasdemir, A., Taskiran, M., & Ayyildiz, N. (2018). Effects of low and high doses of acetylsalicylic acid on penicillin-induced epileptiform activity. *Pharmacological reports : PR*, *70*(5), 885–889. https://doi.org/10.1016/j.pharep.2018.03.002

Tsou, K., Brown, S., Sañudo-Peña, M. C., Mackie, K., & Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*, *83*(2), 393–411. https://doi.org/10.1016/s0306-4522(97)00436-3

Tokarski, K., Zahorodna, A., Bobula, B., & Hess, G. (2002). Comparison of the effects of 5-HT1A and 5-HT4 receptor activation on field potentials

and epileptiform activity in rat hippocampus. *Experimental brain research*, *147*(4), 505–510. https://doi.org/10.1007/s00221-002-1259-6

Türel, C. A., Çelik, H., Torun, İ. E., Çetinkaya, A., & Türel, İ. (2022). The antiinflammatory and electrophysiological effects of fingolimod on penicillin-induced rats. Os efeitos antiinflamatórios e eletrofisiológicos do fingolimode em ratos induzidos pela penicilina. *Arquivos de neuro-psiquiatria*, *80*(12), 1220–1226. https://doi.org/10.1055/s-0042-1758754

Urigüen, L., García-Gutiérrez, M. S., & Manzanares, J. (2011). Decreased GABAA and GABAB receptor functional activity in cannabinoid CB1 receptor knockout mice. *Journal of psychopharmacology*, *25*(1), 105–110. https://doi.org/10.1177/0269881109358204

Venzi, M., David, F., Bellet, J., Cavaccini, A., Bombardi, C., Crunelli, V., & Di Giovanni, G. (2016). Role for serotonin2A (5-HT2A) and 2C (5-HT2C) receptors in experimental absence seizures. *Neuropharmacology*, *108*, 292–304.

Wang, Y., Tan, B., Wang, Y., & Chen, Z. (2021). Cholinergic Signaling, Neural Excitability, and Epilepsy. *Molecules*, *26*(8), 2258. https://doi.org/10.3390/molecules26082258

Wanleenuwat, P., Suntharampillai, N., & Iwanowski, P. (2020). Antibiotic-induced epileptic seizures: mechanisms of action and clinical considerations. *Seizure*, *81*, 167–174. https://doi.org/10.1016/j.seizure.2020.08.012

Yang, Z., Liu, X., Yin, Y., Sun, S., & Deng, X. (2012). Involvement of 5-HT-receptors in the pathogenesis of temporal lobe epilepsy. *European journal of pharmacology*, *685*(1-3), 52–58. https://doi.org/10.1016/j.ejphar.2012.04.011