Indian Journal of Experimental Biology
Vol. 63, January 2025, pp. 25-38
DOI: 10.56042/ijeb.v63i01.9155

Orexinergic and cannabinoid CB1 receptors interaction on genetic absence epilepsy in WAG/Rij rats

Fatma Banu Aycik*, Mustafa Ayyildiz & Erdal Agar

Department of Physiology, Faculty of Medicine, University of Ondokuz, Mayis 55139, Samsun, Türkiye

Received 28 February 2024; revised 15 May 2024

The goal of this study is to clarified the effect of orexinergic system and interaction between orexinergic and cannabinoid systems in WAG/Rij rats. WAG/Rij rats were used to electrocorticogram recordings. 0.6 µg hemopressin, 7.5 µg arachidonyl-2′-chloroethylamide hydrate (ACEA), 0.50 µg AM-251 and also 4, 8, 16 µg doses of OX-A, 6 and 12 µg doses of SB334867 applied and effective doses were determined. After three hours of baseline recording in rats, the effective doses of the substance were applied by intracerebroventricularly, and recording continued for three more hours. Interaction groups were also subjected to the same procedure. Records were analyzed in terms of seizure duration and number, spike number and amplitude. 8 µg OX-A and 12 µg SB334867 significantly decreased the duration and number of seizures, spike number, excluding amplitude (P<0.05). While hemopressin and ACEA decreased absence seizure activity, AM-251 increased it. When application of OX-A+hemopressin, spike wave discharges (SWDs) activity reduced and it was determined that OX-A showed an activity similar to its own effect. Since the effect of the SB334867+hemopressin group was similar to the effect of SB334867 alone, it is thought that the effect of orexins on the cannabinoid pathway may more effective than the hemopressin. In SB334867+AM-251 groups, SWDs activity significantly reduced and the proconvulsant effect of AM-251 was blocked by SB334867. In OX-A+AM-251 group, SWDs activity decreased. This results may show that the orexinergic system is more effective than the cannabinoid system.

Keywords: Orexin-A, SB334867, AM-251, ACEA, Hemopressin

Epilepsy is a common and serious neurological disease that causes recurrent seizures[1]. Although the mechanism of epilepsy is not fully known, factors such as head trauma, bleeding in the brain, some infections in the brain, stroke, tumors in the brain and structural vascular disorders cause epilepsy[2]. Idiopathic epilepsies are characterized by recurrent local or generalized seizures without brain damage[3]. Typical absence epilepsy, which is in the idiopathic generalized epilepsy class, is seen in childhood and constitutes 10% of all pediatric epilepsies[4]. It is generalized form of epilepsy characterized by acute and short-term loss of consciousness with bilateral and symmetrical spike-wave discharges (SWDs) at a frequency of 2-4.5 Hz on electroencephalogram (EEG). Experimental models have been developed to continue absence epilepsy studies in an experimental environment. For this, GAERS and WAG/Rij strains are used[5]. All rats of this strain have spontaneous EEG and generalized spike wave discharges (SWDs), which are key features of absence seizures in humans[6].

Neuropeptides have an important role in modulating seizures and epilepsy[7]. Orexin (OX), a neuropeptide, is involved in various physiological tasks such as energy homeostasis[8], sleep-wake cycle[9], drinking behavior[10], analgesia[11], attention[12], learning[13], and memory[14]. The orexin/hypocretin system was identified in 1998[15]. Sakurai *et al.* named the first isolated peptide from orexin-A (OX-A) and stated that it was 33 amino acids long. Similarly, they defined the second peptide, 28 amino acids long, which causes an increase in intracellular calcium concentration and shows 46% (13/28) homology to the first, as orexin-B (OX-B)[16]. Orexins are released from orexin-containing neurons in the lateral hypothalamic area (LHA)[15]. OX-A and OX-B produce their effects by binding to two types of receptors (GPRCs), orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R)[17]. OX1R has 100-1000 times greater affinity for OX-A, while OX2R has equal affinity for both orexin types[18,19]. Both orexin receptors are probably capable of binding to $G_{i/o}$, G_s and G_q family G proteins; however, this may

*Correspondence:
Phone: +90 362 312 1919
E-mail: fatmabanus@gmail.com

depend on the texture and specific modulation[20]. Orexins play an important role in appetite, energy homeostasis, feeding, arousal and sleep[21,22]. Studies investigating the orexin-epilepsy relationship have conflicting results. Contrary to studies showing that orexins have antiepileptic effects[23,24], it has also been reported that orexins increase the pathogenesis of epilepsy[25-27]. Kortunay *et al*.[28] showed that intracortical application of orexin-A (100 pmol) and orexin-B (100 pmol) increased epileptic activity in cortical penicillin model epileptic adult rats. An increase in EEG total spectrum and tonic-clonic contractions was observed in the extremities of rats treated with orexin-A and B [29].

The cannabinoid system is important because of its role in the central nervous system and neurophysiological events[30]. Cannabinoids have two types of receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2)[31]. CB1 receptors located at the presynaptic terminals of central nervous system (CNS) neurons are highly expressed in limbic structures (amygdala, hippocampus), cerebral cortex, basal ganglia, and certain regions of the midbrain and medulla[32] and are bound to a G protein. The major expression of CB2 receptors is in peripheral immune tissues such as the spleen, lymph nodes, and bone marrow, as well as on B cells, macrophages, and microglia, where activation can induce immunosuppressive responses[33]. The cannabinoid system has an important role in the regulation of seizure activities in the brain[34-36]. The selective CB1 receptor agonist ACEA has an anticonvulsant effect against PTZ-induced seizures in mice[37]. It is known that epileptiform activity is suppressed by cannabinoids and its different mechanism has been suggested. CB1 receptor activation inhibits adenylate cyclase (AC) via $G_{i/o}$ proteins, then production of cyclic adenosine monophosphate (cAMP) is inhibited. When cAMP is reduced, protein kinase (PKA) is inactivated and thus the K^+A channel is stimulated, resulting in K^+ outflow from the cell to the extracellular fluid[38]. In addition to this, it is thought that with CB1 receptor activation, glutaminergic systems are suppressed in a dose-dependent manner, while GABAergic systems are activated[39]. In the absence model, the CB1 receptor agonist R(+)WIN55,212 has been reported to reduce the incidence of dose-dependent SWDs. In addition, it is emphasized that CB1 receptors are involved in synchronization mechanisms within the thalamocortical network and this will increase the possibility of targeting these receptors by new anti-absence drugs[40]. It has been reported that high GABAergic tone in the ventrobasal thalamic nucleus (VBTN) facilitates the creation of SWDs[41]. Infusion of the endogenous cannabinoid anandamide (AEA) and the non-selective CB1 receptor agonist WIN55,212 to the reticular thalamic nucleus (RTN) and ventroposteromedial thalamic nucleus (VPM) reduces the seizures regardless of the focal region, while focal application of the selective CB1 receptor antagonist rimonabant (SR141716A) increase absence seizures[42]. Injection of WIN 55,212-2 to rats prior to PTZ has been shown to reduce phase 4 contraction times[43]. When the cannabinoid CB1 receptor is active, it triggers some pathways; suppression of the L-type Ca^{2+} channel, activation of inward-rectifying K^+ currents[44], reduction of the frequency of excitatory postsynaptic currents (EPSCs)[45], and suppression of neuronal excitotoxicity[46].

Hemopressin (PVNFKFLSH) is a nanopeptide derived from the α chain of hemoglobin[47]. Hemopressin selectively binds to CB1 cannabinoid receptors, but it is unclear whether it is a CB1 receptor agonist or antagonist[48-50]. An *in vitro* study revealed that hemopressin is a selective inverse agonist of the CB1 receptor[49]. This peptide has been shown to signal in a similar effect to rimonabant, the CB1 receptor agonist[49]. Since anorexia, one of the physiological effects of hemopressin was not observed in mutant male mice without CB1R, this effect was expressed to be mediated by CB1R[48]. Hemopressin, acting as a modulator of CB1R receptors, has been shown to enhance oligodendroglia differentiation in neural cultures containing stem cells derived from the subventricular region of newborn mice. These results further suggested that hemopressin, like endocannabinoids, has a physiological function and could be used therapeutically to treat demyelinating diseases[51]. Hemopressin fragment NFKF is shown to decrease the symptoms of pilocarpine-induced epileptic seizures[52]. It has been suggested that hemopressins are present in the adrenal medulla as well as in the catecholaminergic neurons of the central nervous system of rodents and have biological and physiological functions like endocannabinoids[53]. It has been observed that 0.6 μg hemopressin increases penicillin model epileptic activity[54]. It has been clearly stated that the cannabinoid CB1 receptor

system plays a role in epilepsy, but studies investigating the effect of hemopressin on epilepsy are rare. In this respect, it is important to elucidate the effect of hemopressin on epileptic activity.

The effects of orexins on the cannabinoid pathway have been reported in studies. On the other hand, its role in epilepsy is contradictory. Therefore, in this study we hypothesized the role of orexins in epilepsy and their interactions with cannabinoid receptor agonists and antagonists in the absence epilepsy model.

Materials and Methods

Animals

A total of 105 male WAG/Rij (6-8 months) rats were obtained from the Experimental Animal Application and Research Center of the University of Ondokuz Mayis, Samsun, Türkiye. WAG/Rij rats weighing 230-270 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 (2018/44). Animals were divided into groups by random distribution method. Blind system analysis was used during the analysis. Effective doses of 0.50 µg AM251, 7.5 µg ACEA[55]and 0.6 µg Hemopressin[54] obtained from previous studies in our laboratory were taken as reference. Effective doses of orexin-A and SB-334867 were determined based on the literature[29]. Experimental groups were randomly divided into 15 groups as follows; DMSO group (control group); OX-A (4 µg, i.c.v.) group; OX-A (8 µg, i.c.v.) group; OX-A (16 µg, i.c.v.) group; SB-334867 (6 µg, i.c.v.) group; SB-334867 (12 µg, i.c.v.) group; Hemopressin (0.6 µg, i.c.v.) group; AM-251 (0.50 µg, i.c.v.) group; ACEA (7.5 µg, i.c.v.) group; OX-A (8 µg, i.c.v.) + Hemopressin (0.6 µg, i.c.v.); SB-334867 (12 µg, i.c.v.) + Hemopressin (0.6 µg, i.c.v.) group; OX-A (8 µg, i.c.v.) + AM-251 (0.50 µg, i.c.v.) group; SB-334867 (12 µg, i.c.v.) + AM-251 (0.50 µg, i.c.v.) group; OX-A (8 µg, i.c.v.) + ACEA (7.5 µg, i.c.v.) group; SB-334867 (12 µg, i.c.v.) + ACEA (7.5 µg, i.c.v.) group; each group consists of 7 rats.

Substance administration

Cannabinoid CB1 receptor agonist ACEA, cannabinoid CB1 receptor antagonist AM-251, orexin-1 receptor agonist orexin-A, orexin-1 receptor antagonist SB334867 were dissolved in dimethylsulfoxide (DMSO) with saline (DMSO/saline 3:7 v/v), hemopressin was dissolved in serum physiologic (SF) and the doses were administered intracerebroventricularly (i.c.v.). AM-251 and ACEA doses were decided according to Kozan *et al.*[56]. On the other hand, hemopressin dose was determined according to Al-Kaleel *et al.*[57]. While 4, 8 and 16 µg doses of OX-A were used to determine the effective dose, 6 and 12 µg doses of SB334867 were used. The doses of OX-A and SB334867 were chosen from unpublished data in our laboratory.

For surgery, WAG/Rij rats used in the absence model of epilepsy were anesthetized with ketamine/xylazine (90/10 mg/kg, i.p.). Animals were placed in the stereotaxic device. Four holes were drilled with manual drills, three for electrode placement and one for i.c.v. injection (The electrode coordinates for WAG/Rij rats are as follows; frontal region coordinates; 3 mm anterior and 4 mm lateral, parietal region coordinates; 6 mm posterior and 4 mm lateral, intracerebroventricular (i.c.v.) coordinates; 1.5 mm lateral and 1.1 mm posterior to the bregma and 4.2 mm ventral to the surface of the skull[58]). After inserting the external cannula into the hole drilled for i.c.v. injections, both screws and the i.c.v. cannula was fixed to the skull with the help of dental acrylic and waited for drying. After the surgical procedure, the animals were kept for 1 week for recovery period.

Electrocorticography (ECoG) recordings

Animals were connected to the ECoG recording system (PowerLab, 4/SP, AD Instruments, Castle Hill, Australia) on the experiment day *via* cable. For the absence model, ECoG recordings were started at the same time of the day (09.00 AM.) and injection was performed after 180 min of basal recording. The recording was taken for another 180 min after the injection. After the experiments were finished, the seizure clusters in epileptiform activity were manually selected, and the ECoG recordings were analyzed in 20 min portions by calculating the duration of SWDs, the number of SWDs, the number of spikes and their amplitude using Chart v7.0.3 (ADInstruments, Australia) software. The data obtained in the first 180 min were divided into 20 min intervals and the average of this section was calculated. Each 20 min interval obtained after the injection was compared with this average in percentage terms.

Statistical analysis

Recorded electrophysiological data were statistically compared with SPSS (Statistical Package

for Social Sciences) 17.0. The normality of the data was evaluated with the Shapiro-Wilk test. To compare multiple groups with normal distribution One-way analysis of variance (ANOVA) was used, and Bonferroni *post-hoc* test was used to determine the differences between these groups. Kruskal Wallis test and then *post-hoc* Dunn test were performed in multiple groups that did not comply with the normal distribution. Values of experimental groups in graphics and texts were shown as mean±standard error (SEM), $P<0.05$ value was considered statistically significant.

Results

Effect of DMSO on absence epilepsy model in WAG/Rij rats

For the control group, DMSO used as a solvent in WAG/Rijs was administered as i.c.v. 3 h after the start of ECoG recording. Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 6.0±0.3, 38.3±3.7 sec, 212.6±7.0, 0.8367±0.0311 mV in the 100th min after administration of DMSO, respectively. (Fig. 1)

Effect of orexin-A on absence epilepsy model

In order to determine the effective dose of OX-A in WAG/Rij rats, doses of 4, 8 and 16 µg were applied. Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 3.5±0.2, 15.5±0.6 sec, 73.5±1.1, 0.7312±0.0142 mV in the 100th min after administration of OX-A (4 µg), respectively. Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 1.2±0.2, 4.4±1.0 sec, 6.8±2.0, 0.7507±0.0389 mV in the 100th min after administration of OX-A (8 µg), respectively. Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 1,5±0,4, 2,3±1,2 sec, 11,1±6,7, 0,7817±0,0189 mV in the 100th min after administration of OX-A (16 µg), in turn in order. When all these doses of orexin-A were compared with the control group, a statistically significant decrease was observed in total numbers, mean durations, spike numbers of SWDs ($P<0.05$) (Fig. 1) and amplitude of SWDs did not change statistically ($P>0.05$). However, the most statistically significant reduction was seen at 8 µg and 16 µg doses compared to the control group. When we compared these two groups among themselves, lower dose of orexin-A (8 µg) was accepted as the effective dose since there was no statistical difference ($P=0.96$).

Effect of SB334867 on absence epilepsy model

To determine the effective dose of SB-334867 in WAG/Rij rats, doses of 6 and 12 µg were administered (Fig. 2). Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 4.0±0.1, 24.6±1.7 sec, 156.5±12.0, 0.9381±0.0171 mV in the 100th min after administration of SB334867 (6 µg), respectively. When this group was compared to the control group, there was no statistically significant difference in any of the parameters ($P=2.68$). Total numbers, mean durations, spike

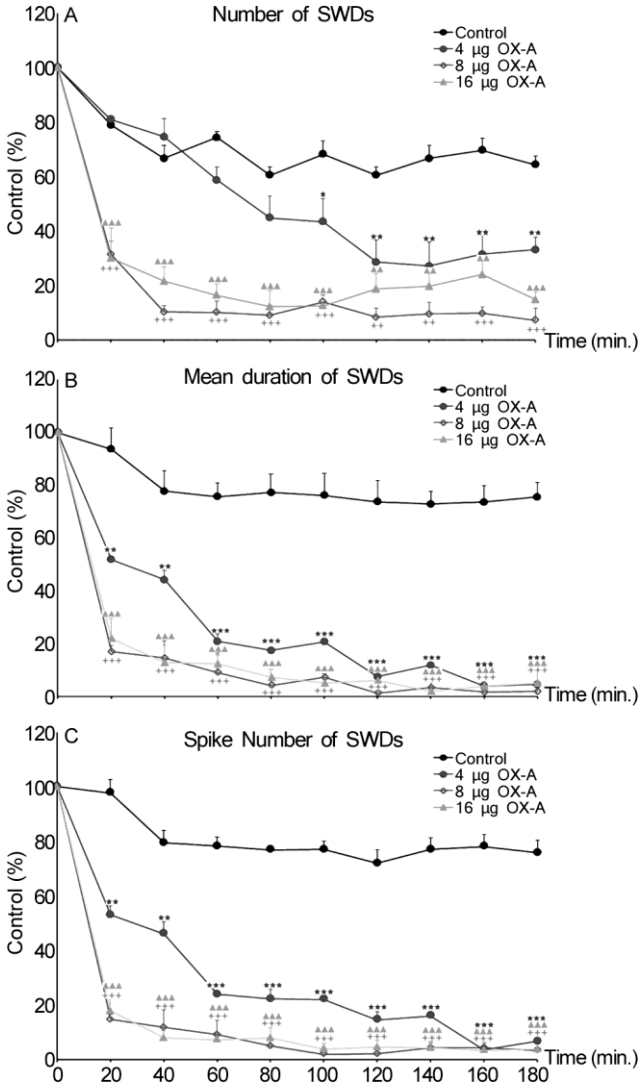

Fig. 1 — Comparison of the % control of (A) SWDs numbers (number of SWDs % control±SEM), (B) SWDs duration (duration of SWDs % control±SEM), (C) spike numbers (duration of SWDs % control±SEM) of the 4 µg, 8 µg, and 16 µg groups of Orexin-A with the control group. When these doses of Orexin-A were compared with the control group, a statistically significant decrease was observed in all three parameters. (⋆: comparison of 4 µg OX-A to control group, +: comparison of 8 µg OX-A to control group, ▲: comparison of 16 µg OX-A to control group)[⋆=$P<0.05$, ⋆⋆= $P<0.01$, ⋆⋆⋆= $P<0.001$]

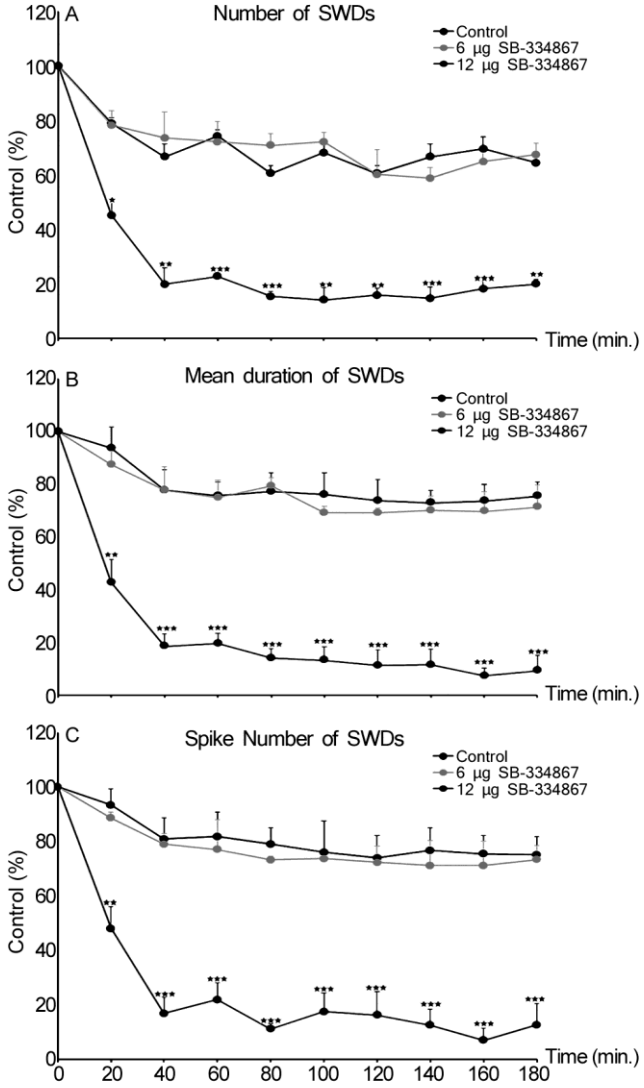

Fig. 2 — Comparison of the % control of **A.** SWDs numbers (number of SWDs % control±SEM), **B.** SWDs duration (duration of SWDs % control±SEM), **C.** spike numbers (duration of SWDs % control±SEM) of the 6 µg and 12 µg SB-334867 according to control group. A statistically significant decrease was observed when 12 µg SB-334867 was compared with the control group) [⋆=P<0.05, ⋆⋆= P<0.01, ⋆⋆⋆= P<0.001]

numbers and mean amplitudes of SWDs were 1.6±0.6, 7.4±2.7 sec, 47.2±6.4, 0.6832±0.0162 mV in the 100th min after administration of SB334867 (12 µg), respectively. 12 µg of SB334867 was accepted as the effective dose as it statistically significantly reduced total numbers, mean durations, spike numbers of SWDs (P>0.05).

Effect of 0.6 µg hemopressin on absence epilepsy model

The administration of 0.6 µg hemopressin (i.c.v.) causes a decrease in PTZ-induced epileptic activity

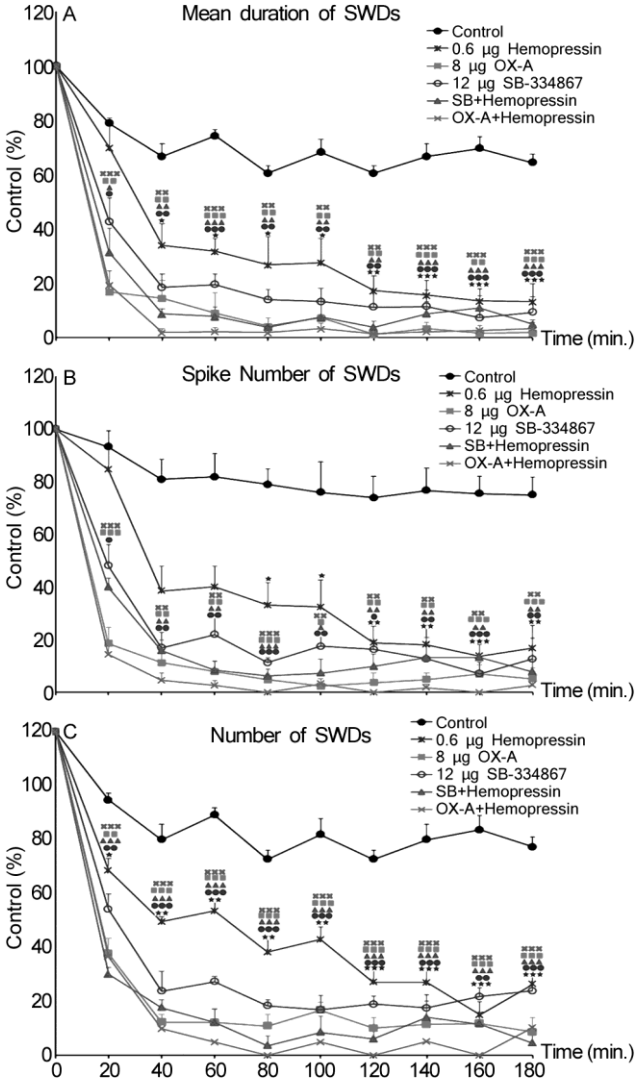

Fig. 3 — Comparison of the % control of **A.** SWDs numbers (number of SWDs % control±SEM), **B.** SWDs duration (duration of SWDs % control±SEM), **C.** spike numbers (duration of SWDs % control±SEM) of the Hemopressin, OX-A, SB334867, SB334867+Hemopressin and OX-A+Hemopressin group according to the control group. Compared with the control group, a statistically significant decrease was observed in all groups (⋆: comparison of 0.6 µg hemopressin to control group, ●: comparison of 12 µg SB334867 to control group, ■: comparison of 8 µg OX-A to control group, ▲: comparison of SB34867+Hemopressin group to control group, **x**: comparison of OX-A+ Hemopressin group to control group))[⋆=P<0.05, ⋆⋆= P<0.01, ⋆⋆⋆= P<0.001]

(Al-Kaleel *et al*., 2023). Total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 1.8±0.2, 10.6±3.5 sec, 69.3±9.6, 0.830±0.0247 mV in the 100th min after administration of hemopressin (0.6 µg), respectively. 0.6 µg hemopressin reduced total numbers, mean durations, spike numbers of SWDs 40 min after injection, significantly (Fig. 3) (P>0.05).

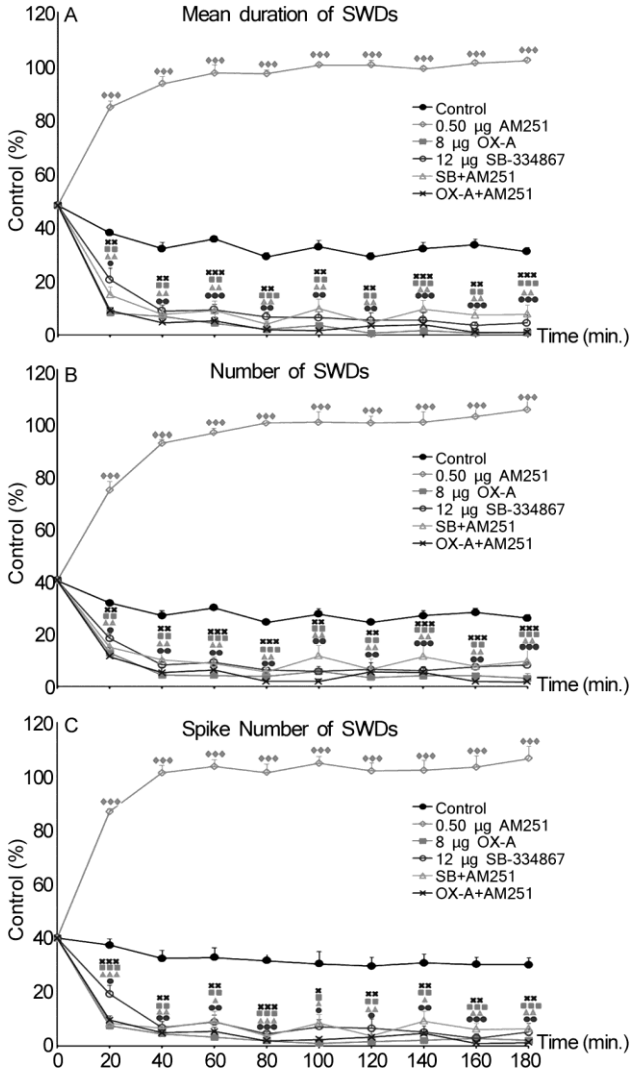

Fig. 4 — Comparison of the % control of **A.** SWDs numbers (number of SWDs % control±SEM), **B.** SWDs duration (duration of SWDs % control±SEM), **C.** spike numbers (duration of SWDs % control±SEM) of the AM-251, OX-A, SB334867, SB-334867 + AM-251 and OX-A+AM-251 group according to the control group. Compared with the control group, a statistically significant decrease was observed in all groups except the AM251 group (♦: comparison of 0.50 µg AM251 to control group, ■: comparison of 8 µg OX-A to control group, ●: comparison of 12 µg SB334867 to control group, ▲: comparison of SB34867+AM251 group to control group, **x**: comparison of OX-A+ AM251 group to control group)[♦=P<0.05, ♦♦= P<0.01, ♦♦♦= P<0.001]

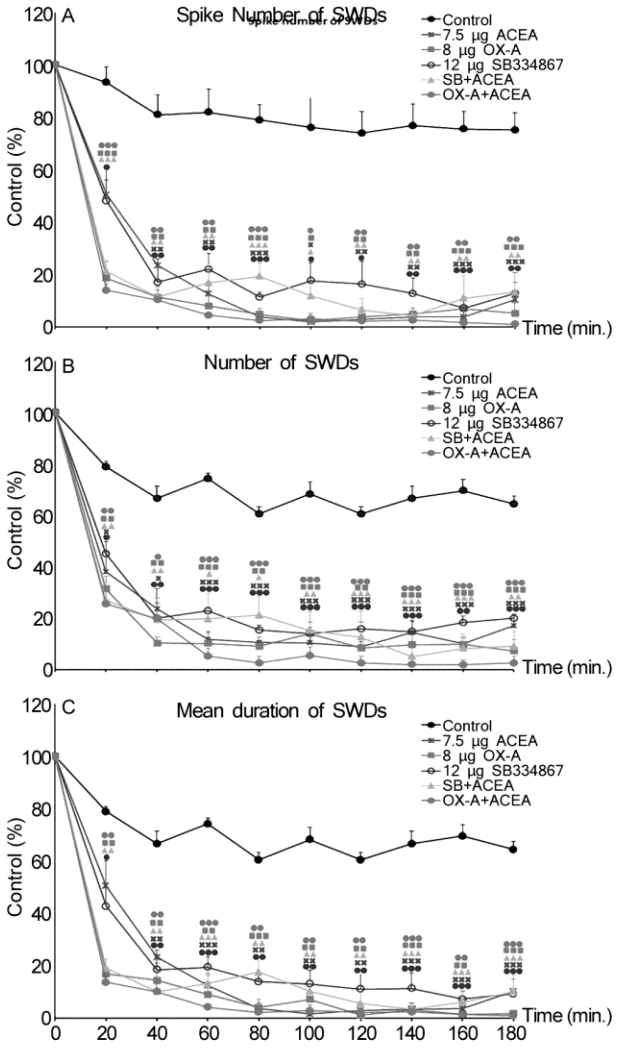

Fig. 5 — Comparison of the % control of **A.** SWDs numbers (number of SWDs % control±SEM), **B.** SWDs duration (duration of SWDs % control±SEM), **C.** spike numbers (duration of SWDs % control±SEM) of the ACEA, OX-A, SB334867, SB-334867 + ACEA and OX-A+ACEA group according to the control group. Compared with the control group, a statistically significant decrease was observed in all groups (**x**: comparison of 7.5 µg ACEA to control group, ■: comparison of 8 µg OX-A to control group, ●: comparison of 12 µg SB334867 to control group, ▲: comparison of SB34867+ACEA group to control group, :● comparison of OX-A+ACEA group to control group [**x** = P<0.05, **x x** =P<0.01, **x x x** = P<0.001]

Role of cannabinoid receptor CB1 on absence epilepsy model

CB1 receptor antagonist AM-251 increased the total number, mean duration and spike number of SWDs after injection. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 13±0.3, 67.3±4.2 sec, 498.6±2.2, 0.9189±0.0556 mV in the 100th min after administration of AM-251 (0.50 µg), respectively (Fig. 4). CB1 receptor agonist ACEA significantly decreased the total number, mean duration and spike number of SWDs 20 min after injection compared to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 2.1±0.4, 6.8±2.2 sec, 13.4±4.5, 0.9258±0.0319 mV in the 100th min after administration of ACEA (7.5 µg), respectively (Fig. 5).

Interaction between orexinergic system and hemopressin

Hemopressin was injected 10 min after OX-A. The total number, mean duration and spike number of SWDs are reduced after 20 min injection of hemopressin according to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 0.3±0.2, 2±1.2 sec, 10.6±6.7, 0.8226±0.0478 mV in the 100th min after co-administration of hemopressin with OX-A, respectively (Fig. 3). Hemopressin was injected 10 min after SB334867. The total number, mean duration and spike number of SWDs are declined after 20 min injection of hemopressin compared to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 0.7±0.4, 5.0±3.2 sec, 22.2±8.5, 0.862±0.0306 mV in the 100th min after co-administration of hemopressin with SB334867, respectively (Fig. 3).

Interaction between orexinergic system and cannabinoid CB1 receptor

AM-251 was injected 10 min after OX-A. The total number, mean duration and spike number of SWDs are reduced after 20 min injection of AM-251 according to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 0.3±0.2, 2±1.2 sec, 17.3±5.6, 0.8912±0.0249 mV in the 100th min after co-administration of AM-251 with OX-A, respectively (Fig. 4). AM-251 was injected 10 min after SB334867. The total number, mean duration and spike number of SWDs are declined after 20 min injection of AM-251 compared to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 2.2±0.8, 10.2±4.3 sec, 69.6±9.7, 0.7907±0.0459 mV in the 100th min after co-application of AM-251 with SB334867, respectively (Fig. 4). ACEA was injected 10 min after OX-A. The total number, mean duration and spike number of SWDs are reduced after 20 min injection of ACEA according to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 0.4±0.2, 2±1.3 sec, 16.2±8.7, 0,8456±0,0371 mV in the 100th min after co-administration of ACEA with OX-A, respectively (Fig. 5). ACEA was injected 10 min after SB334867. The total number, mean duration and spike number of SWDs are reduced after 20 min injection of ACEA compared to control group. The total numbers, mean durations, spike numbers and mean amplitudes of SWDs were 1.2±0.5, 6.4±2.7 sec, 44.8±10.7, 0.8266±0.0383 mV in the 100th min after co-application of ACEA with SB334867, respectively (Fig. 5).

Discussion

This study aimed to show the relationship between the orexinergic system and the cannabinoid CB1 receptor on absence epilepsy in WAG/Rij rats. Firstly we determined the effective dose of OX-A, an orexin-1 receptor agonist, and SB334867, an orexin-1 receptor antagonist. The electrophysiological results showed that the application of 8 µg OX-A and 12 µg SB334867 displayed an anticonvulsant effect without changing the mean amplitude of SWDs. SWDs activity was decreased in the presence of CB1 receptor agonist 7.5 µg ACEA and 0.6 µg hemopressin while CB1 receptor antagonist 0.50 µg AM251 increased it. Studies are showing that orexins are associated with neurodegenerative processes. A study reported that OX-A (i.c.v.) administration had neuroprotective effects in the MPTP-induced C57BL/6 mouse model of Parkinson's disease[59]. Microinjection of OX-A in the hippocampus of A53T mice ameliorated performance in these hippocampus-dependent memory tasks[60]. It is said that orexins may be useful for slowing the development of multiple sclerosis (MS) by inhibiting neuroinflammation and subsequent neurodegeneration[61]. Δ9-THC, a cannabinoid, displayed important therapeutic potential for the treatment of neurodegenerative diseases, like Alzheimer′s disease (AD). Some studies reported that its ability to interfere with Aβ aggregation in vitro, influence Aβ fibrils formation and aggregation, induce the removal of intracellular Aβ, and block the inflammatory response[62].

Studies have shown conflicting results about the effect of orexins on the pathogenesis of epilepsy [63]. Orexins have antiepileptic activity in some studies[23,24,64], while in others they exacerbate the pathogenesis of epilepsy[25-27,65]. Intracortical (i.c.) injection of 100 pmol OX-A and 100 pmol OX-B in rats increased epileptic activity in the penicillin model epilepsy[28]. In another study, an increase in EEG total spectrum and tonic-clonic contractions was observed in the extremities of rats treated with OX-A and OX-B[29]. Contrary to this study, 100 nM OX-A suppresses epileptic activity in a bicululine-induced epilepsy model[23]. Considering the results of the presented study, it was seen that OX-A reduced SWDs activity in genetic absence epilepsy. Orexin-1

system deficiency may contribute to the complex pathophysiology of recurrent generalized tonic-clonic (GTC) seizures and status epilepticus (SE)[24]. However, its mechanism has not been fully elucidated. OX-A inhibited epileptiform activity in the bicululine model epilepsy, and the existence of conflicting results about orexin may be due to different experimental epilepsy models[23]. These findings support the view that the orexinergic system may exert different effects in response to different pathophysiological conditions such as epilepsy and that its antiepileptic activity may result from the difference in the experimental model used. The balance between the glutamatergic and GABAergic systems is disrupted during epileptic activity. Some studies reported that orexin increases the release of glutamate[66-68] and GABA[69], in contrast others decreases the release of glutamate[70] and GABA[71]. In the presented study, OX-A may have an anticonvulsant effect by directly acting on neuron depolarization by decreasing the levels of glutamate, an excitatory neurotransmitter. Orexin receptor antagonist, SB334867, reduces seizure intensity and convulsive stage, while orexin receptor stimulation does not improve seizure intensity compared to the PTZ group[72]. Akbari[72] explained this controversy, it was stated that orexin activates the histamine and histamine-secreting tuberomamillary nucleus (TMN)[73] in the posterior hypothalamus and it exerts an antiepileptic effect in the PTZ model following deep brain stimulation in the posterior hypothalamus[74]. Since OX2R is the main orexinergic receptor in TMN and its activation leads to histamine release, investigation of the involvement of OX2R may eliminate this discrepancy[72]. OX1R protein levels were lower in the thalamus of 6-7 month old WAG/Rij compared to 25 day old WAG/Rij and 6-7 month old non-epileptic wistar rats[75]. OX1R levels decrease with increasing age, and the formation of SWDs is characterized by a decrease in OX1R protein levels[75]. Orexin-induced depolarization may facilitate T-type Ca^{2+} channel inactivation in the ventrobasal nucleus or reticular thalamic nuclei, thereby restricting the development of SWDs[75]. According to some *in vitro* experimental studies, orexins have been reported to cause depolarization of neurons[76] and increase in discharge frequency of neurons[77,78]. Orexins can exert these effects by increasing the flow of sodium and creating direct stimulating effects on neurons[79], activating the sodium-calcium exchanger pump[78], increasing the flow of calcium[80], or reducing the flow of potassium[81]. SB-334867 (10 µg/rat, i.c.v.) reduced behavior-related seizures in the PTZ model in rats[82]. SB334867 reduce the intensity of PTZ-induced convulsions[72]. Our results are compatible with these studies and may show that SB334867, an orexin receptor antagonist, reduces epileptic activity by increasing extracellular calcium influx or decreasing potassium flux, activating the sodium-calcium exchange pump.

Cannabinoid system has an important role in regulation of seizure activity in brain[34-36]. 0.25 µg AM-251 (i.s.v.) increased the frequency of epileptiform activity and showed status epilepticus-like activities characterized by burst spikes[83]. The CB1 receptor agonist R(+)WIN55,212 dose-dependently decreased the incidence of SWDs in WAG/Rij rats with absence epilepsy, and decreased CB1 receptor expression in the reticular thalamic nucleus may be associated with the development of SWDs[40]. Infusion of the endogenous cannabinoid anandamide (AEA) and the non-selective CB1 receptor agonist WIN55,212 to the reticular thalamic nucleus (NRT) and ventroposteromedial thalamic nucleus (VPM) reduces absence seizures regardless of the focal region, while the selective CB1 receptor antagonist, rimonabant (SR141716A), has been shown to increase absence seizures when applied focally[42]. Application of low doses of AM-251 (0.125 µg and 0.25 µg) in WAG/Rij rats decreased the number and duration of SWDs and the number of spikes in each SWDs, while 0.50 µg AM-251 (i.c.v.) increased all three parameters and 7.5 µg ACEA (i.c.v.) reduced absence seizures[55]. In the present study, in parallel with the study of Aygun *et al*.[55], it was observed that 7.5 µg ACEA decreased SWDs activity in WAG/Rij rats, while 0.50 µg AM-251 increased SWDs activity. Cannabinoids are known to suppress epileptiform activity through different pathways. As a result of CB1 receptor activation, adenylate cyclase (AC) is inhibited and cAMP production is prevented. Protein kinase A (PKA) is also inactivated when cAMP is reduced. Thus, the K^+A channel is stimulated, and K^+ comes out of the cell into the extracellular fluid[38]. These channels are particularly important in the homeostatic regulation of membrane excitability. Potassium channels are involved in the treatment of diseases such as epilepsy and brain ischemia[84]. Stimulation of $G_{i/o}$ proteins by the CB1 receptor causes direct inhibition of N-type or

P/Q-type Ca^{2+} channels and directly inward rectifying K^+ activation[38]. The entry of Ca^{2+} into the cell is important in the occurrence of epileptic activity[85]. This causes the secretion of excitatory amino acids, especially glutamate.

Hemopressin is the first peptide ligand specifically classified for the CB1 cannabinoid receptor[49]. Hemopressins are predominantly found in both the adrenal medulla and the catecholaminergic neurons of the rodents' central nervous system and have the same biological and physiological importance as endocannabinoids[53]. Although hemopressin has an inverse agonist/antagonistic activity, hemopressin forms (i.e. RVD-hemopressin, also called pepcan-12) display type 1 and type 2 cannabinoid receptor agonistic/allosteric activity[86]. The effects of synthetic CB1R and CB2R agonists occur through the activation of receptor-associated $G_{i/o}$ proteins. While adenylate cyclase activity and cAMP production are inhibited, a decrease in Ca^{2+} and an increase in K^+ conductance are observed[87]. Hemopressin acted as a CB1R inverse agonist[49]. Hemopressin has an antagonist effect on the high CB1R activity, like cannabinoid inverse agonist SR141716[86]. The pharmacological binding properties of hemopressin peptides contribute to their physiological effects that occur in different pathological conditions[86]. When hemopressin administered centrally or systemically, hemopressin reduced night-time food intake dose-dependently, and this effect is regulated through CB1R[48]. 50 μg/kg (i.p.) and 500 μg/kg (i.p.) doses of hemopressin has an antinociceptive effect[49]. Hemopressin (i.c.v.) application extend memory retention and improves memory formation in mice[88]. Transient receptor potential channels (TRP channels) have a role in chemical transduction aimed by endocannabinoids[89]. Hemopressin (i.c.v.) causes anxiogenic-like effects by activating TRPV1 receptors[90]. Hemopressin raised Na+/K + -ATPase activity after 1 min of application, whereas inhibiting it after 15 min of treatment[91]. Capsazepine, a TRPV1 antagonist, inhibited the fast raised of Na+/K + -ATPase activity induced by hemopressin. The late inhibitory effect of hemopressin on Na+/K + -ATPase activity was prevented by the CB1R antagonist AM251[91]. There are few studies investigating the effect of hemopressin on epileptiform activity. 0.6 μg hemopressin (i.c.v.) was accepted as the effective dose because it increased epileptic activity in penicillin model epilepsy[54]. In a study conducted with a cerebral ischemia/reperfusion mouse model and investigating the neuroprotective effect of cannabinoid agonists and antagonists, 1 mg/kg hemopressin (i.p.) suppressed the neuroprotective effect of the cannabinoid receptor agonist ACEA[92]. In the PTZ kindling model, 0.030 μg and 0.6 μg hemopressin reduced the total duration, total number, and number of spikes of spike-wave discharges[57]. In this study, 0.6 μg of hemopressin appeared to act as a CB1 receptor agonist, reducing the activity of SWDs recorded from animals with absence epilepsy.

The OX-1 receptor and CB1 receptors both belong to the GPRC superfamily and their distribution in the brain, particularly in the hippocampus, may overlap[93]. In experiments using electron microscopy, it has been determined that CB1 and OX-1 receptors are close to each other because they are located near the plasma membrane and form a heterodimer[94]. There is a relationship between OX-1 receptors and cannabinoid receptors[95,96]. Orexin receptor signaling can trigger the production of 2-arachidonyl glycerol (2-AG), which activates CB1 receptors[70,97]. In the present study, interaction groups were created to reveal the relationship between both systems. The central administration of RDV-hemopressin (α) (a N-terminally extended form of hemopressin) has investigated on anxiety like behavior and food intake, and they have shown that RVD-hp(α) stimulated anxiolytic and orexinergic effects possibly related to reduced norepinephrine and orexin-A and POMC signaling, in the hypothalamus[98]. 20.1 nmol mouse VD-hemopressin α (i.c.v.) increased both non-rapid eye movement (NREM) sleep in the first 2 h section and EEG delta (0.5–4 Hz) activity. (m)VD-HPα promotes NREM sleep via the CB1 cannabinoid receptor to probably activate VLPO GABAergic neurons, but inactivates the LH orexinergic, LC noradrenergic, and TMN histaminergic neurons[99]. In OX-A+hemopressin group, OX-A showed an activity similar to its own effect. Although the anticonvulsant effect of the interaction group was high, statistical significance was not observed. Hemopressin and orexin effect cannabinoid CB1 receptors[86,97]. The effect observed in the interaction group is similar as that of OX-A′s effect. The effect of OX-A on cannabinoid pathways may be more than hemopressin. When effective doses of OX-A and AM-251 were applied together, the activity of SWDs decreased. In addition to this group, when AM-251 was given before OX-A, AM-251 did not show its

own proconvulsant effect until the 80th min, but it also abolished the anticonvulsant effect of OX-A. After 80 min, OX-A continued its anticonvulsant activity by showing its own effect. From this point of view, OX-A might be showing its effect through another mechanism besides its effect on cannabinoidergic pathways. OX receptor signaling in the dorsal raphe nucleus has been reported to inhibit glutamate release to serotonergic neurons[70]. Orexins regulate phospholipases, ion channels and protein kinases and trigger activation of various signaling pathways[100]. The ameliorative effects of OX-A on the spatial learning and memory deficits of PTZ-induced epileptic rats are facilitated through OX-1R-mediated ERK1/2-mediated neurogenesis in the dentate gyrus[64]. Antinociceptive effect of OX-A is markedly reduced when co-administered with AM-251[97]. In OX-A+ACEA group, the anticonvulsant activities of both substances did not combine to cause a synergistic effect. OX1 receptor signaling generates diacyl glyceol (DAG) via the phospholipase C (PLC) pathway, DAG is hydrolyzed to 2-arachidonyl glycerol (2-AG) by the diacyl glycerol lipase enzyme (DGL) and 2-AG stimulates CB1 receptors. 2-AG stimulates ERK activation[101]. Endocannabinoids (EC) are produced postsynaptically and mediate retrograde inhibitory effects on presynaptic terminals via the CB1 receptor[87]. The analgesic effects of orexin on the ventrolateral periaqueductal gray matter are also mediated by endocannabinoid production and release[97]. OX1R-mediated 2-AG biosynthesis and activation of CB1 receptors, which are targets of endocannabinoids in the brain, underlie the diverse effects of OX-A in the brain[102,103]. OX-A may also act on the cannabinoid system and create an antiepileptic response. Since the effect of the SB334867+hemopressin group was similar to that of SB334867 alone, it confirmed that the effect of orexins on the cannabinoid system may be greater than the hemopressin. In the SB334867 and AM-251 groups, SWDs activity was significantly reduced and the proconvulsant effect of AM-251 was blocked by SB334867. The orexinergic system dominates the cannabinoidergic system. SB334867 not only blocks orexin-A-induced action, but also potentiates cannabinoid CB2R function in cAMP and MAPK signaling pathways[104]. In a study of antinociception induced by chemical stimulation of the lateral hypothalamus in rats, SB334867 and AM-251 administered into the ventrolateral periaqueductal gray matter dose-dependently blocked carbochol-induced antinociception, the pain-modulatory role of the lateral hypothalamus was mediated by the interaction between orexinergic systems in the periaqueductal gray matter[105].

In the present study were examined the effects of OX receptor agonist orexin-A and orexin receptor antagonist SB334867 on absence epilepsy, these substances reduced seizures. The cannabinoid CB1 receptor antagonist AM-251 (at very low dose, 100 pg/kg) has an anticonvulsant effect when administered with the CB1 receptor agonist ACEA[106], while at higher doses it has a proconvulsant effect[56]. At the same time, low dose (0.25 and 0.125 µg) AM-251 (i.c.v) reduces spike number and SWDs activity in PTZ model epilepsy[57]. Previous studies have suggested that cannabinoid compounds may have opposite effects at low and high doses[57]. For example, tetrahydrocannabinol (THC), which acts as a CB1 receptor agonist, exerts an anticonvulsant effect at low doses by reducing glutamate release from presynaptic excitatory axon terminals[107]. However, high doses of THC may have a pro-convulsant effect by lowering the seizure threshold by stimulating CB1 receptors on GABAergic axon terminals[108]. A study suggested that when a CB1 antagonist was administered for a certain period of time, it could prevent the development of seizures[109] , and it was emphasized that the short-term plasticity mechanism was mediated by endocannabinoids, such as depolarization-induced suppression of inhibition (DSI). When CB1 antagonist blocks these receptors, it prevents DSI and reduces seizure susceptibility[110]. The possible mechanism of the anticonvulsant effect of low dose AM-252 is that it can block the CB1 receptor in inhibitory interneurons, prevent interneuron inhibition, and increase the inhibition/excitation ratio[57]. Cannabinoids act through multiple pathways such as ion channel and protein kinase in the cell. Therefore, the existence of different effects of orexins may be due to the use of different intracellular pathways depending on dose, just like the CB1 receptor antagonist AM-251. In this study, the role and relationship of orexinergic and cannabinoid systems in absence epilepsy were investigated by electrophysiological method.

Conclusion

The role of orexin in absence epilepsy is that it reduces SWDs activity. While hemopressin and ACEA decreased absence seizure activity, AM-251 increased

it. When the interaction group data are examined, it can be said that there is a relationship between the cannabinoid system and the orexinergic system. This interaction may possibly lead to changes in the level of receptor gene expression. However, molecular, gene expression studies and protein levels studies are needed to elucidate the underlying mechanisms. Results obtained from different experimental animals may lead to new clinical phase studies.

Conflict of interest

The authors declare no conflict of interest.

References

1 Jia C, Zhang R, Wei L, Xie J, Zhou S, Yin W, Hua X, Xiao N, Ma M&Jiao H. Investigation of the mechanism of tanshinone IIA to improve cognitive function via synaptic plasticity in epileptic rats. *Pharm Biol*. (2023);61(1):100-10.

2 Loscher W. Animal models of intractable epilepsy. *Prog Neurobiol*. (1997);53(2):239-58.

3 Crunelli V&Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci*. (2002); 3(5):371-82.

4 Garzon P, Lemelle L&Auvin S. [Childhood absence epilepsy: An update]. *Arch Pediatr*. (2016);23(11):1176-83.

5 Medvedeva TM, Sysoeva MV, Luttjohann A, van Luijtelaar G & Sysoev IV. Dynamical mesoscale model of absence seizures in genetic models. *PLoS One*. (2020); 15(9): e0239125.

6 Sarkisova KY&Kulikov MA. Behavioral characteristics of WAG/Rij rats susceptible and non-susceptible to audiogenic seizures. *Behav Brain Res*. (2006);166(1):9-18.

7 Kovac S & Walker MC. Neuropeptides in epilepsy. *Neuropeptides*. (2013);47(6):467-75.

8 Fernandez-Rilo AC, Forte N, Palomba L, Tunisi L, Piscitelli F, Imperatore R, Di Costanzo A, Di Marzo V & Cristino L. Orexin induces the production of an endocannabinoid-derived lysophosphatidic acid eliciting hypothalamic synaptic loss in obesity. *Mol Metab*. (2023);72:101713.

9 Kniazkina M&Dyachuk V. Does EGFR Signaling Mediate Orexin System Activity in Sleep Initiation? *Int J Mol Sci*. (2023);24(11).

10 Kunii K, Yamanaka A, Nambu T, Matsuzaki I, Goto K & Sakurai T. Orexins/hypocretins regulate drinking behaviour. *Brain Res*. (1999);842(1):256-61.

11 Mobarakeh JI, Takahashi K, Sakurada S, Nishino S, Watanabe H, Kato M&Yanai K. Enhanced antinociception by intracerebroventricularly and intrathecally-administered orexin A and B (hypocretin-1 and -2) in mice. *Peptides*. (2005);26(5):767-77.

12 Fadel J & Burk JA. Orexin/hypocretin modulation of the basal forebrain cholinergic system: Role in attention. *Brain Res*. (2010);1314:112-23.

13 Telegdy G&Adamik A. The action of orexin A on passive avoidance learning. Involvement of transmitters. *Regul Pept*. (2002);104(1-3):105-10.

14 Akbari E, Naghdi N & Motamedi F. The selective orexin 1 receptor antagonist SB-334867-A impairs acquisition and consolidation but not retrieval of spatial memory in Morris water maze. *Peptides*. (2007);28(3):650-6.

15 de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM & Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*. (1998);95(1):322-7.

16 Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richarson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ&Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. (1998);92(5):1 page following 696.

17 Sheibani M, Shayan M, Khalilzadeh M, Ghasemi M & Dehpour AR. Orexin receptor antagonists in the pathophysiology and treatment of sleep disorders and epilepsy. *Neuropeptides*. (2023);99:102335.

18 Sakurai T. The role of orexin in motivated behaviours. *Nat Rev Neurosci*. (2014);15(11):719-31.

19 Willie JT, Chemelli RM, Sinton CM&Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci*. (2001);24:429-58.

20 Kukkonen JP. Physiology of the orexinergic/hypocretinergic system: a revisit in 2012. *Am J Physiol Cell Physiol*. (2013);304(1):C2-32.

21 Bonnavion P&de Lecea L. Hypocretins in the control of sleep and wakefulness. *Curr Neurol Neurosci Rep*. (2010); 10(3):174-9.

22 Sakurai T. The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci*. (2007);8(3):171-81.

23 Doreulee N, Alania M, Vashalomidze G, Skhirtladze E & Kapanadze T. Orexinergic system and pathophysiology of epilepsy. *Georgian Med News*. (2010)(188):74-9.

24 Rejdak K, Papuc E, Grieb P&Stelmasiak Z. Decreased cerebrospinal fluid hypocretin-1 (orexin A) in patients after repetitive generalized tonic-clonic seizures. *Epilepsia*. (2009);50(6):1641-4.

25 Goudarzi E, Elahdadi Salmani M, Lashkarbolouki T & Goudarzi I. Hippocampal orexin receptors inactivation reduces PTZ induced seizures of male rats. *Pharmacol Biochem Behav*. (2015);130:77-83.

26 Ng MC. Orexin and Epilepsy: Potential Role of REM Sleep. *Sleep*. (2017);40(3).

27 Roundtree HM, Simeone TA, Johnson C, Matthews SA, Samson KK&Simeone KA. Orexin Receptor Antagonism Improves Sleep and Reduces Seizures in Kcna1-null Mice. *Sleep*. (2016);39(2):357-68.

28 Kortunay S, Erken HA, Erken G, Genc O, Sahiner M, Turgut S & Turgut G. Orexins increase penicillin-induced epileptic activity. *Peptides*. (2012);34(2):419-22.

29 Erken HA, Erken G, Genc O, Kortunay S, Sahiner M, Turgut G & Turgut S. Orexins cause epileptic activity. *Peptides*. (2012);37(1):161-4.

30 Agar E. The role of cannabinoids and leptin in neurological diseases. *Acta Neurol Scand*. (2015); 132(6):371-80.
31 Ferre S, Kofalvi A, Ciruela F, Justinova Z & Pistis M. Targeting corticostriatal transmission for the treatment of cannabinoid use disorder. *Trends Pharmacol Sci*. (2023); 44(8):495-506.
32 Tsou K, Brown S, Sanudo-Pena MC, Mackie K & Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*. (1998);83(2):393-411.
33 Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G & Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem*. (1995);232(1):54-61.
34 Deshpande LS, Sombati S, Blair RE, Carter DS, Martin BR & DeLorenzo RJ. Cannabinoid CB1 receptor antagonists cause status epilepticus-like activity in the hippocampal neuronal culture model of acquired epilepsy. *Neurosci Lett*. (2007);411(1):11-6.
35 Luszczki JJ, Czuczwar P, Cioczek-Czuczwar A & Czuczwar SJ. Arachidonyl-2'-chloroethylamide, a highly selective cannabinoid CB1 receptor agonist, enhances the anticonvulsant action of valproate in the mouse maximal electroshock-induced seizure model. *Eur J Pharmacol*. (2006); 547(1-3):65-74.
36 Wallace MJ, Blair RE, Falenski KW, Martin BR & DeLorenzo RJ. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J Pharmacol Exp Ther*. (2003);307(1):129-37.
37 Bahremand A, Nasrabady SE, Shafaroodi H, Ghasemi M & Dehpour AR. Involvement of nitrergic system in the anticonvulsant effect of the cannabinoid CB(1) agonist ACEA in the pentylenetetrazole-induced seizure in mice. *Epilepsy Res*. (2009);84(2-3):110-9.
38 Kyrou I, Valsamakis G&Tsigos C. The endocannabinoid system as a target for the treatment of visceral obesity and metabolic syndrome. *Ann N Y Acad Sci*. (2006);1083: 270-305.
39 Feigenbaum JJ, Bergmann F, Richmond SA, Mechoulam R, Nadler V, Kloog Y & Sokolovsky M. Nonpsychotropic cannabinoid acts as a functional N-methyl-D-aspartate receptor blocker. *Proc Natl Acad Sci U S A*. (1989);86(23):9584-7.
40 van Rijn CM, Gaetani S, Santolini I, Badura A, Gabova A, Fu J, Watanabe M, Cuomo V, van Luijtelaar G, Nicoletti F & Ngomba RT. WAG/Rij rats show a reduced expression of CB(1) receptors in thalamic nuclei and respond to the CB(1) receptor agonist, R(+)WIN55,212-2, with a reduced incidence of spike-wave discharges. *Epilepsia*. (2010); 51(8):1511-21.
41 McCormick DA&Bal T. Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci*. (1997);20:185-215.
42 Citraro R, Russo E, Ngomba RT, Nicoletti F, Scicchitano F, Whalley BJ, Calignano A&De Sarro G. CB1 agonists, locally applied to the cortico-thalamic circuit of rats with genetic absence epilepsy, reduce epileptic manifestations. *Epilepsy Res*. (2013);106(1-2):74-82.
43 Zirotti Rosenberg A, Mendez-Ruette M, Gorziglia M, Alzerreca B, Cabello J, Kaufmann S, Rambousek L, Iturriaga Jofre A, Wyneken U&Lafourcade CA. Behavioral and Molecular Responses to Exogenous Cannabinoids During Pentylenetetrazol-Induced Convulsions in Male and Female Rats. *Front Mol Neurosci*. (2022);15:868583.
44 Busquets-Garcia A, Bains J&Marsicano G. CB(1) Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. *Neuropsychopharmacology*. (2018);43(1):4-20.
45 Bhaskaran MD & Smith BN. Cannabinoid-mediated inhibition of recurrent excitatory circuitry in the dentate gyrus in a mouse model of temporal lobe epilepsy. *PLoS One*. (2010);5(5):e10683.
46 Kokona D&Thermos K. Synthetic and endogenous cannabinoids protect retinal neurons from AMPA excitotoxicity in vivo, via activation of CB1 receptors: Involvement of PI3K/Akt and MEK/ERK signaling pathways. *Exp Eye Res*. (2015);136:45-58.
47 Rioli V, Gozzo FC, Heimann AS, Linardi A, Krieger JE, Shida CS, Almeida PC, Hyslop S, Eberlin MN&Ferro ES. Novel natural peptide substrates for endopeptidase 24.15, neurolysin, and angiotensin-converting enzyme. *J Biol Chem*. (2003);278(10):8547-55.
48 Dodd GT, Mancini G, Lutz B&Luckman SM. The peptide hemopressin acts through CB1 cannabinoid receptors to reduce food intake in rats and mice. *J Neurosci*. (2010); 30(21):7369-76.
49 Heimann AS, Gomes I, Dale CS, Pagano RL, Gupta A, de Souza LL, Luchessi AD, Castro LM, Giorgi R, Rioli V, Ferro ES&Devi LA. Hemopressin is an inverse agonist of CB1 cannabinoid receptors. *Proc Natl Acad Sci U S A*. (2007);104(51):20588-93.
50 Li XH, Lin ML, Wang ZL, Wang P, Tang HH, Lin YY, Li N, Fang Q&Wang R. Central administrations of hemopressin and related peptides inhibit gastrointestinal motility in mice. *Neurogastroenterol Motil*. (2016); 28(6): 891-9.
51 Xapelli S, Agasse F, Grade S, Bernardino L, Ribeiro FF, Schitine CS, Heimann AS, Ferro ES, Sebastiao AM, De Melo Reis RA & Malva JO. Modulation of subventricular zone oligodendrogenesis: a role for hemopressin? *Front Cell Neurosci*. (2014);8:59.
52 de Araujo CB, Heimann AS, Remer RA, Russo LC, Colquhoun A, Forti FL&Ferro ES. Intracellular Peptides in Cell Biology and Pharmacology. *Biomolecules*. (2019);9(4).
53 Hofer SC, Ralvenius WT, Gachet MS, Fritschy JM, Zeilhofer HU & Gertsch J. Localization and production of peptide endocannabinoids in the rodent CNS and adrenal medulla. *Neuropharmacology*. (2015);98:78-89.
54 Aygun H, Arslan G, Sen E, Ayyildiz M & Agar E. Hemopressin increases penicillin-induced epileptiform activity in rats. *Bratisl Lek Listy*. (2020);121(1):37-42.
55 Aygün H, Arslan G, Ayyildiz M&Agar E. The Effect of CB1 Cannabinoid Receptor Antagonist AM-251 on Spike Wave Discharges in WAG/Rij Rat. *Acta Physiol*. (2015); 215:46-.
56 Kozan R, Ayyildiz M & Agar E. The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. *Epilepsia*. (2009);50(7): 1760-7.
57 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*. (2023);475(6):719-30.

58 Przewlocka B, Lason W, Turchan J, de Bruin N, van Luijtelaar G, Przewlocki R&Coenen A. Anatomical and functional aspects of mu opioid receptors in epileptic WAG/Rij rats. *Epilepsy Res*. (1998);29(2):167-73.

59 Liu MF, Xue Y, Liu C, Liu YH, Diao HL, Wang Y, Pan YP & Chen L. Orexin-A Exerts Neuroprotective Effects via OX1R in Parkinson's Disease. *Front Neurosci*. (2018);12:835.

60 Stanojlovic M, Pallais JP, Lee MK & Kotz CM. Pharmacological and chemogenetic orexin/hypocretin intervention ameliorates Hipp-dependent memory impairment in the A53T mice model of Parkinson's disease. *Mol Brain*. (2019);12(1):87.

61 Ten-Blanco M, Flores A, Cristino L, Pereda-Perez I&Berrendero F. Targeting the orexin/hypocretin system for the treatment of neuropsychiatric and neurodegenerative diseases: From animal to clinical studies. *Front Neuroendocrinol*. (2023);69:101066.

62 Ferrisi R, Gado F, Ricardi C, Polini B, Manera C & Chiellini G. The Interplay between Cannabinoid Receptors and Microglia in the Pathophysiology of Alzheimer's Disease. *J Clin Med*. (2023);12(23).

63 Berhe DF, Gebre AK&Assefa BT. Orexins role in neurodegenerative diseases: From pathogenesis to treatment. *Pharmacol Biochem Behav*. (2020);194:172929.

64 Zhao X, Zhang R, Tang S, Ren Y, Yang W, Liu X & Tang J. Orexin-A-induced ERK1/2 activation reverses impaired spatial learning and memory in pentylenetetrazol-kindled rats via OX1R-mediated hippocampal neurogenesis. *Peptides*. (2014);54:140-7.

65 Ni LY, Zhu MJ, Song Y, Liu XM & Tang JY. Pentylenetetrazol-induced seizures are exacerbated by sleep deprivation through orexin receptor-mediated hippocampal cell proliferation. *Neurol Sci*. (2014);35(2):245-52.

66 John J, Wu MF, Kodama T&Siegel JM. Intravenously administered hypocretin-1 alters brain amino acid release: an in vivo microdialysis study in rats. *J Physiol*. (2003); 548 (Pt 2):557-62.

67 Kodama T&Kimura M. Arousal effects of orexin-A correlate with GLU release from the locus coeruleus in rats. *Peptides*. (2002);23(9):1673-81.

68 van den Pol AN, Gao XB, Obrietan K, Kilduff TS & Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J Neurosci*. (1998);18(19):7962-71.

69 Martin G, Fabre V, Siggins GR&de Lecea L. Interaction of the hypocretins with neurotransmitters in the nucleus accumbens. *Regul Pept*. (2002);104(1-3):111-7.

70 Haj-Dahmane S&Shen RY. The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus serotonin neurons through retrograde endocannabinoid signaling. *J Neurosci*. (2005);25(4):896-905.

71 Thorpe AJ, Doane DF, Sweet DC, Beverly JL & Kotz CM. Orexin A in the rostrolateral hypothalamic area induces feeding by modulating GABAergic transmission. *Brain Res*. (2006);1125(1):60-6.

72 Akbari N, Salmani ME, Goudarzvand M, Lashkar Boluki T, Goudarzi I & Abrari K. Unilateral Hypothalamus Inactivation Prevents PTZ Kindling Development through Hippocampal Orexin Receptor 1 Modulation. *Basic Clin Neurosci*. (2014);5(1):66-73.

73 Ishizuka T, Murotani T&Yamatodani A. Modanifil activates the histaminergic system through the orexinergic neurons. *Neurosci Lett*. (2010);483(3):193-6.

74 Nishida N, Huang ZL, Mikuni N, Miura Y, Urade Y & Hashimoto N. Deep brain stimulation of the posterior hypothalamus activates the histaminergic system to exert antiepileptic effect in rat pentylenetetrazol model. *Exp Neurol*. (2007);205(1):132-44.

75 Celli R&Luijtelaar GV. The Orexin System: A Potential Player in the Pathophysiology of Absence Epilepsy. *Curr Neuropharmacol*. (2022);20(7):1254-60.

76 Shirasaka T, Miyahara S, Kunitake T, Jin QH, Kato K, Takasaki M&Kannan H. Orexin depolarizes rat hypothalamic paraventricular nucleus neurons. *Am J Physiol Regul Integr Comp Physiol*. (2001);281(4):R1114-8.

77 Burlet S, Tyler CJ&Leonard CS. Direct and indirect excitation of laterodorsal tegmental neurons by Hypocretin/Orexin peptides: implications for wakefulness and narcolepsy. *J Neurosci*. (2002);22(7):2862-72.

78 Eriksson KS, Sergeeva O, Brown RE & Haas HL. Orexin/ hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. *J Neurosci*. (2001);21(23):9273-9.

79 Liu RJ, van den Pol AN&Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci*. (2002);22(21):9453-64.

80 Allard JS, Tizabi Y, Shaffery JP, Trouth CO & Manaye K. Stereological analysis of the hypothalamic hypocretin/orexin neurons in an animal model of depression. *Neuropeptides*. (2004);38(5):311-5.

81 Ivanov A&Aston-Jones G. Hypocretin/orexin depolarizes and decreases potassium conductance in locus coeruleus neurons. *Neuroreport*. (2000);11(8):1755-8.

82 Kordi Jaz E, Moghimi A, Fereidoni M, Asadi S, Shamsizadeh A&Roohbakhsh A. SB-334867, an orexin receptor 1 antagonist, decreased seizure and anxiety in pentylenetetrazol-kindled rats. *Fundam Clin Pharmacol*. (2017);31(2):201-7.

83 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*. (2013);19(4):222-8.

84 Misonou H. Homeostatic regulation of neuronal excitability by K(+) channels in normal and diseased brains. *Neuroscientist*. (2010);16(1):51-64.

85 Zhou X, Chen Z, Xiao L, Zhong Y, Liu Y, Wu J & Tao H. Intracellular calcium homeostasis and its dysregulation underlying epileptic seizures. *Seizure*. (2022); 103:126-36.

86 Heimann AS, Dale CS, Guimaraes FS, Reis RAM, Navon A, Shmuelov MA, Rioli V, Gomes I, Devi LL&Ferro ES. Hemopressin as a breakthrough for the cannabinoid field. *Neuropharmacology*. (2021);183.

87 Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M & Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev*. (2009);89(1):309-80.

88 8Zhang RS, He Z, Jin WD&Wang R. Effects of the cannabinoid 1 receptor peptide ligands hemopressin, (m) RVD-hemopressin (alpha) and (m)VD-hemopressin (alpha) on memory in novel object and object location recognition tasks in normal young and Abeta1-42-treated mice. *Neurobiol Learn Mem*. (2016);134 Pt B:264-74.

89 Muller C, Morales P & Reggio PH. Cannabinoid Ligands Targeting TRP Channels. *Front Mol Neurosci*. (2018);11:487.

90 Fogaca MV, Sonego AB, Rioli V, Gozzo FC, Dale CS, Ferro ES & Guimaraes FS. Anxiogenic-like effects induced by hemopressin in rats. *Pharmacol Biochem Behav*. (2015);129:7-13.

91 Sampaio LS, Taveira Da Silva R, Lima D, Sampaio CL, Iannotti FA, Mazzarella E, Di Marzo V, Vieyra A, Reis RA&Einicker-Lamas M. The endocannabinoid system in renal cells: regulation of Na(+) transport by CB1 receptors through distinct cell signalling pathways. *Br J Pharmacol*. (2015);172(19):4615-25.

92 Ma L, Jia J, Niu W, Jiang T, Zhai Q, Yang L, Bai F, Wang Q & Xiong L. Mitochondrial CB1 receptor is involved in ACEA-induced protective effects on neurons and mitochondrial functions. *Sci Rep*. (2015);5:12440.

93 Zhu F, Wang XQ, Chen YN, Yang N, Lang SY, Zuo PP, Zhang JT & Li RS. Changes and overlapping distribution in the expression of CB1/OX1-GPCRs in rat hippocampus by kainic acid-induced status epilepticus. *Brain Res*. (2015);1597:14-27.

94 Hilairet S, Bouaboula M, Carriere D, Le Fur G & Casellas P. Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. *J Biol Chem*. (2003); 278(26):23731-7.

95 Kim HJJ, Zagzoog A, Smolyakova AM, Ezeaka UC, Benko MJ, Holt T & Laprairie RB. In vivo Evidence for Brain Region-Specific Molecular Interactions Between Cannabinoid and Orexin Receptors. *Front Neurosci*. (2021);15:790546.

96 Turunen PM, Jantti MH&Kukkonen JP. OX1 orexin/hypocretin receptor signaling through arachidonic acid and endocannabinoid release. *Mol Pharmacol*. (2012);82(2):156-67.

97 Ho YC, Lee HJ, Tung LW, Liao YY, Fu SY, Teng SF, Liao HT, Mackie K & Chiou LC. Activation of orexin 1 receptors in the periaqueductal gray of male rats leads to antinociception via retrograde endocannabinoid (2-arachidonoylglycerol)-induced disinhibition. *J Neurosci*. (2011);31(41):14600-10.

98 Recinella L, Chiavaroli A, Ferrante C, Mollica A, Macedonio G, Stefanucci A, Dimmito MP, Dvoracsko S, Tomboly C, Brunetti L, Orlando G & Leone S. Effects of central RVD-hemopressin(alpha) administration on anxiety, feeding behavior and hypothalamic neuromodulators in the rat. *Pharmacol Rep*. (2018);70(4):650-7.

99 Xie JF, Wang LX, Ren WT, Wang C, Gao JX, Chen HL, Zhao XQ, Ren YL, Xie YP, Shao YF&Hou YP. An alpha-hemoglobin-derived peptide (m)VD-hemopressin (alpha) promotes NREM sleep via the CB(1) cannabinoid receptor. *Front Pharmacol*. (2023);14:1213215.

100 Wang C, Wang Q, Ji B, Pan Y, Xu C, Cheng B, Bai B & Chen J. The Orexin/Receptor System: Molecular Mechanism and Therapeutic Potential for Neurological Diseases. *Front Mol Neurosci*. (2018);11:220.

101 Kukkonen JP&Leonard CS. Orexin/hypocretin receptor signalling cascades. *Br J Pharmacol*. (2014);171(2):314-31.

102 Cristino L, Luongo L, Imperatore R, Boccella S, Becker T, Morello G, Piscitelli F, Busetto G, Maione S&Di Marzo V. Orexin-A and Endocannabinoid Activation of the Descending Antinociceptive Pathway Underlies Altered Pain Perception in Leptin Signaling Deficiency. *Neuropsychopharmacology*. (2016);41(2):508-20.

103 Morello G, Imperatore R, Palomba L, Finelli C, Labruna G, Pasanisi F, Sacchetti L, Buono L, Piscitelli F, Orlando P, Di Marzo V & Cristino L. Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. *Proc Natl Acad Sci U S A*. (2016);113(17):4759-64.

104 Raich I, Rebassa JB, Lillo J, Cordomi A, Rivas-Santisteban R, Lillo A, Reyes-Resina I, Franco R&Navarro G. Antagonization of OX(1) Receptor Potentiates CB(2) Receptor Function in Microglia from APP(Sw/Ind) Mice Model. *Int J Mol Sci*. (2022);23(21).

105 Esmaeili MH, Reisi Z, Ezzatpanah S & Haghparast A. Functional interaction between orexin-1 and CB1 receptors in the periaqueductal gray matter during antinociception induced by chemical stimulation of the lateral hypothalamus in rats. *Eur J Pain*. (2016);20(10):1753-62.

106 Gholizadeh S, Shafaroodi H, Ghasemi M, Bahremand A, Sharifzadeh M&Dehpour AR. Ultra-low dose cannabinoid antagonist AM251 enhances cannabinoid anticonvulsant effects in the pentylenetetrazole-induced seizure in mice. *Neuropharmacology*. (2007);53(6):763-70.

107 Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K & Freund TF. Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci*. (2006); 26(21):5628-37.

108 Hoffman AF&Lupica CR. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J Neurosci*. (2000);20(7):2470-9.

109 Dodd GT, Worth AA, Hodkinson DJ, Srivastava RK, Lutz B, Williams SR & Luckman SM. Central functional response to the novel peptide cannabinoid, hemopressin. *Neuropharmacology*. (2013);71:27-36.

110 Chen K, Neu A, Howard AL, Foldy C, Echegoyen J, Hilgenberg L, Smith M, Mackie K&Soltesz I. Prevention of plasticity of endocannabinoid signaling inhibits persistent limbic hyperexcitability caused by developmental seizures. *J Neurosci*. (2007);27(1):46-58.