E. Malkov et al. Effects of Endocannabinoid-Related Compounds on the Activity of Septal and Hippocampal Neurons in a Model of Kainic Neurotoxicity: Study Ex Vivo

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Abstract. The goal of the work was to study the effect of the cannabinoid receptor agonist WIN55,212-2 and the cannabinoid type 1 receptor antagonist AM251 on electrophysiological changes in the hippocampus and the medial septal region (MS) induced by the intracerebral administration of excitotoxin kainic acid. Kainate injected into the right brain ventricle provoked persistent seizures (status epilepticus, SE) in all rats. A morphological analysis of the right hippocampus performed one month after the SE revealed the death of neurons, which was most pronounced in the hilus of the dentate gyrus and in the CA3a field of the dorsal hippocampus. In brain slices taken one month after the SE, the spontaneous activity of MS neurons and population EPSP (pEPSP) in the CA1 field of the hippocampus evoked by the stimulation of Shaffer collaterals (SC) was recorded; the changes in the activity were compared with the activity in slices of healthy animals injected with normal saline (“control slices”). It was found that the activity in MS slices from the brain of animals injected with kainic acid (“kainate slices”) was almost twice higher than in the control. After the application of WIN55,212-2, the frequency of discharges in the control did not change, whereas in kainate slices, the level of neuronal activity decreased to the control value. The application of AM251 led to an increase in the frequency of discharges in the control and its decrease in kainate slices. The registration of pEPSPs in the hippocampal slices revealed a twofold increase in the responses to SC stimulation in kainate slices compared with those in the control, i.e., an abrupt increase in neuronal excitability. A tendency for a decrease in excitability after the application of WIN55,212-2 and, conversely, for its increase by the action of AM251 was noted in evoked responses in the hippocampal kainate slices. Our results allow to assume the protective impact of cannabinoid agonist WIN55,212-2 on neuronal activity in the medial septum and hippocampus that disturbed by neurotoxic kainate influence.

Keywords: medial septum, hippocampus, neuronal activity, pEPSPs, WIN55,212-2, AM251

Abbreviations: ACSF - artificial cerebrospinal fluid; eCBs - endogenous cannabinoids; FJB - Fluoro-Jade B; FV - fiber volley; KA - kainic acid; MS - medial septal region; pEPSP - population EPSP; SC - Shaffer collaterals; SE - status epilepticus; TLE - temporal lobe epilepsy

Introduction. The neurotoxic effects of the agonist of ionotropic glutamate receptors of kainic acid (KA) cause a hyperexcitation of neurons and acute seizures, which subsequently result in the impairment of the brain, primarily the hippocampus. This property of KA is used for the creation of models of temporal lobe epilepsy (TLE) (Ben-Ari et al., 1979; Morimoto et al., 2004). The glutamate excitotoxicity is observed as a consequence of acute brain traumas and intoxication as well as during the development of neurodegenerative diseases, including TLE. So far, there are no universal drugs suppressing seizures and the seizure-induced death of neurons. One of promising approaches to the suppression or prevention of seizures as well as subsequent cell death and the development of epilepsy can be the activation of the endogenous cannabinoid system as a natural homeostatic modulator (Katona, Freund, 2008; Kano et al., 2009; Soltesz et al., 2015).

Endogenous cannabinoids (eCBs) are a group of signaling lipid molecules synthesized in neuronal membranes from precursors, which produce their effect on the CNS through the activation of cannabinoid CB1 and CB2 receptors. CB1 receptors are found in many brain regions, in particular, the hippocampus and one of the structures of the basal forebrain, the medial septal region (MS), which includes the medial septal nucleus and the nucleus of the diagonal band of Broca (Bisogno et al., 1999). The major eCBs, two signaling molecules: 2-arachidonoylglycerol (2-AG) and N-arachidonylethanolamide (anandamide), are synthesized and released from postsynaptic cellular sites and act on presynaptically localized CB1 receptors whose activation limits the release of mediators. Thus, eCBs accomplish the retrograde synaptic communication and neuromodulation in the brain (Alger, 2002; Wilson, Nicoll, 2002; Freund et al., 2003). The release of eCBs...
occurs most often in response to prolonged neuronal excitation, which induces an intensive entry of Ca\(^{2+}\) into the cell (Matsuda et al., 1990; Munro et al., 1993); an increase in its intracellular content to a pathological level (Freund et al., 2003) usually leads to the destruction of neurons. Under these conditions, the responsive release of eCBs and the activation of CB1 receptors, which leads to the inhibition of potential-dependent Ca\(^{2+}\) channels (Di Marzo et al., 1998; Felder and Glass, 1998), i.e., limits the entry of Ca\(^{2+}\) into the cell, is an adaptive brain response that attenuates excitotoxic consequences.

The eCB system is involved not only in the regulation of neuronal excitability (Soltesz et al., 2015) but also in the neuronal protection under the influence of toxic agents and destructions (Karanian et al., 2005; Khaspekov et al., 2004; Bobrov et al., 2008; Soltesz et al., 2015; Vinogradova, van Rijn, 2015; Shubina et al., 2015). In particular, its protective function manifests itself markedly in glutamate excitotoxicity (Hansen et al., 2001; Marsicano et al., 2003; Khaspekov et al., 2004; Maroof et al., 2013; Soltesz et al., 2015). In this case, eCBs accomplish neuromodulation in different brain structures “on demand”, depending on the spatial, temporal, and cellular specificity of activated neuronal networks (Alger, 2002; Wilson, Nicoll, 2002; Esch et al., 2006). It should be noted that the seizure activity creates ideal conditions for the synthesis of eCBs (Hauser, Hesdorffer, 1990; Raza et al., 2001).

The hippocampus is the main brain structure where a TLE seizure focus is formed. Other brain regions can also significantly contribute to its development. It has been shown in TLE models that a part of the basal forebrain, the MS, affects the development of seizure activity in the hippocampus (Miller et al., 1994; Colom et al., 2006; Popova et al., 2008; Butuzova, Kitchigina, 2008; Astasheva et al., 2015). The hippocampus and MS are a unified septohippocampal system with two-way communications. The hippocampus contains a large number of CB1 receptors on different cell types, both glutamatergic (Kawamura et al., 2006; Monory et al., 2006) and GABAergic (Tsou et al., 1998); they are particularly abundant in the molecular layer of dentate gyrus and CA3 field (Tsou et al., 1998; Egertova, Elphick, 2000). The mossy cells of the hilus of dentate gyrus, which send glutamatergic projections to dentate fascia, contain the greatest number of CB1 receptors (Monory et al., 2006).

The MS is a region containing a heterogeneous population of neurons producing different mediators such as acetylcholine (Brashear et al., 1986; Gritti et al., 1993; Zaborzsky et al., 1999), GABA (Brashear et al., 1986; Kiss et al., 1990; Gritti et al., 1993), and glutamate (Sotty et al., 2003; Hajszan et al., 2004). MS cells of different neurochemical nature interact with each other through local communications to form a complex neuronal network (Lenthin, Frochter, 1989; Gao et al., 1995; Brauer et al., 1998; Henderson et al., 2001, 2004; Muñson et al., 2003; Wu et al., 2003). It was also found that a great number of CB1 receptors are expressed in the MS (Herkenham et al., 1991; Malleux, Vanderhaeghen, 1992; Matsuda et al., 1993; Romero et al., 1997 Berrendero et al., 1998; Marsicano, Lutz, 1999; Gonzalez et al., 2002; Hrabovszky et al., 2012). However, their role in the functioning of MS remains still obscure.

The goal of the present work was to elucidate how cannabinoid drugs (cannabinoid receptor agonist WIN55,212-2 and the CB1 receptor antagonist AM251) affect the electrical activity in slices of MS and hippocampus taken from healthy animals and KA-injected animals that underwent status epilepticus (SE).

**Methods**

The study was conducted in accordance with the ethical principles formulated in the Helsinki Declaration on the care and use of laboratory animals and the Regulations of the European Parliament (86/609/EC). Electrophysiological experiments were carried out on slices of the MS and the hippocampus from the brain of rats (males weighing 215–255 g; n = 10). The morphological analysis of the hippocampal tissue after SE was performed on the same rats.

**Surgical operations and SE induction**

A week prior to the beginning of experiments, a surgical operation on anesthetized animals was performed. Tiletamine-zolazepam (“Zoletil”, 18 mg/kg, i.m.) was used as the general anesthesia, and xylazine (12 mg/kg, i.m.) served as a myorelaxant. By using the atlas of stereotaxic coordinates (Paxinos, Watson, 1998), guide cannules were implanted above the right brain ventricles of all rats for the injection of pharmacological preparations (AP = -0.9, L = 1.5, H = 3.5).

A week after the surgery, either the neurotoxin KA (0.6 µg/1 µl, n=6) or the physiological saline (n=4) in the same volume was injected to awake animals. The injection of KA led as a rule to the emergence of behavioral seizures (SE); to animals that did not develop seizures after the first injection of KA, the epileptogen was injected again at a half dose. After a month, MS and the septal pole of the right hippocampus were withdrawn from the brain for the electrophysiological experiments. The remaining part of the right hippocampus was used in histological studies to estimate the state of the hippocampal tissue.

**Experiments ex vivo**

Animals were decapitated, the brains were withdrawn and placed in a solution of artificial cerebrospinal fluid (ACSF) containing NaCl 126 mM, KCl 3.50 mM, NaHPO\(_4\) 1.25 mM, NaHCO\(_3\) 25 mM, CaCl\(_2\) 2.00 mM, MgCl\(_2\) 1.30 mM, glucose 10 mM, pH 7.4. The fluid was aerated with carbogen (98% O\(_2\)/5% CO\(_2\)) and cooled to 4°C. Frontal slices of the MS area and parasagittal slices of the hippocampus 350 µm thick were prepared using a NVSL Vibroslicer (WPI, USA). Slices were placed in an incubation chamber with two-side superfusion (10 ml/min) at room temperature for 2 h. The activity of single neurons in the MS area was recorded using ACSF-filled borosilicate microelectrodes with a resistance of 1.9-2.2
In the hippocampal slices (CA1 area), population EPSPs (pEPSPs) of pyramidal neurons evoked by Shaffer collaterals (SC) stimulation were recorded by ACSF-filled borosilicate electrodes with a resistance of 1.2-1.5 mΩ. For SC stimulation, bipolar tungsten electrodes were used; stimuli were delivered at a frequency of 0.1 Hz, and the intensity of stimuli varied from 50 to 300 μA. To access fiber excitability and estimate the synaptic strength in the hippocampus, the input-output (I-O) dependences were measured (Norris, Scheff, 2009). The relative number of activated CA3 afferents in each slice was assessed by plotting average fiber volley (FV) amplitudes against stimulation intensity. Averaged EPSP slope measures were then plotted against their corresponding FV amplitudes to estimate the strength of existing CA3-CA1 synaptic contacts. Fiber excitability and synaptic strength curves for each slice were fit with a sigmoidal equation of the form:

\[ Y = \frac{A_{\text{max}}}{1 + e^{\left(\frac{x-x_c}{b}\right)}} \]

where \( A_{\text{max}} \) – the maximal amplitude of the distribution, \( b \) – the distribution slope, \( x \) – the stimulus intensity (or FV amplitude), and \( x_c \) – the stimulus intensity (or FV amplitude) required for half-maximal response amplitude.

Fitted parameters were then compared across slices using Mann-Whitney U test.

The synthetic cannabinoid agonist (R)-{+}[2,3-Dihydro-5-methyl-3\{4-morpholinyl\}-methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-1-naphthalenyl)methanone (WIN55,212-2; 1μM; Sigma) and the CB1 receptor antagonist/inverse agonist N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251; 2μM; Sigma), were used to activate and block CB1 receptor-mediated responses, respectively. Both these compounds were introduced into the perfusion medium.

Morphological control

One month after the administration of kainate, morphological changes in the dorsal hippocampus were analyzed. After fixation in 4% paraformaldehyde (48h at 4°C) and cryoprotection in a gradient of sucrose (10% and 20% sucrose at 4°C for 24h each) brains were rapidly frozen in the vapor phase of liquid nitrogen and stored at -80°C. Coronal sections (15 μm) were cut with cryostat at -19°C (Thermo Shandon Cryotome E, Thermo Scientific, USA) and collected on gelatin-coated slides for subsequent histochemical stainings.

Neurodegeneration was revealed by Nissl staining using a standard protocol. Slide-mounted sections were submerged in bi-distilled water with acetate buffer for 5 min and stained in fresh 0.1% cresyl violet for 5-8 min until the desired depth of staining was achieved. The Nissl stained slides were then dehydrated through graded ethanols, cleared in xylene, and coverslipped with Eukitt (Fluka, Germany) mounting medium. Bright field images were acquired on a Leica DM6000B microscope (Leica Microsystems, Germany) with a Leica DFC490 camera. All tissue sections were photographed under identical conditions.

In the Nissl-stained sections of right hippocampus, neuronal quantification was carried out in the dentate hilus, CA3a (counting frames 300×300 μm), CA3b and CA1 (counting frames 400×300 μm) fields at level corresponding to AP=-4.52 of the Paxinos and Watson (1998) atlas. At least four different sections were evaluated from each animal. All analyses were carried out using ImageJ software (1.43u, USA) by an investigator who was blind to experimental group.

Fluoro-Jade B (FJB) staining was used to visualize the degenerative neurons in dorsal hippocampus (Schmued, Hopkins, 2000). Samples were placed in 0.06% potassium permanganate solution for 20 min to insure consistent background suppression and stained in the dark with a 0.0001% solution of FJB in 0.1% acetic acid vehicle for 30 min. Then, slices were washed with distilled water with some drops of acetate buffer, dried at 50°C for 10–15 min and immersed into xylene for at least a min before coverslipping with Eukitt (Fluka, Germany) mounting medium. FJB staining were examined in the right hippocampus at levels corresponding to AP=3.3 to -5.2 of the Paxinos and Watson (1998) atlas under fluorescent microscope Leica DM6000B microscope (Leica Microsystems, Germany) with a Leica DFC490 camera using fluorescein isothiocyanate (FITC) filter sets.

An decrease in the number of neurons in the hippocampus and the presence of late signs of neuronal degeneration were considered to result from the neurotoxic effect of KA and subsequent epileptogenesis (Morimoto et al., 2004).

Statistical analysis.

Data are presented as the mean±SEM. The significance of differences between the control and kainate groups was analyzed using the Mann-Whitney U test. The differences within groups were estimated using the Wilcoxon W test. Differences at p<0.05 were considered as significant.

Results

Morphological analysis

A month after the intracerebral administration of KA, changes in the morphological state of the hippocampal tissue were observed (Fig. 1). In the dorsal hippocampus, a significant decrease in the cell number in the hilus and a lesser decrease in the CA3a field were revealed by the Nissl stain (from a partial injury of cells to the almost complete degeneration of the pyramidal layer, depending on the intensity of SE in different animals) (Fig. 1A). The data of the quantitative analysis of morphological changes in the hippocampus are given in Table 1.

Experiments with FJB stain, which allow to detect irreversible neurodegenerative changes, revealed positive labeling in the subiculum, hilus of dentate gyrus, and CA3a field of the dorsal hippocampus (Fig. 1B).
However, it should be noted that the application of the CB1 receptor antagonist AM251 led to oppositely directed changes in the FV intensity, and the resulting I-O curve for each slice was fit with a sigmoidal equation (Table 3; Fig. 2B,C). Averaged pEPSP slope measures were then plotted against their corresponding FV amplitudes (Fig. 2B, center, right). The degree of the excitability of hippocampal neurons after the stimulation of SC was estimated from the slope of the front of pEPSP at different levels of fiber activation, which is described by the sigmoidal function as well. In this case, a higher slope of the curve, was the indicator of an excitability in this communication system (CA3-CA1) (see Table 3).

In our experiments, it was revealed that stimulus intensity sufficient for half-maximal fiber response was significantly lower in kainate slices compared with control ones (p<0.01) (Fig. 2B, left), suggesting higher excitability on presynaptic level. The lower curve slope in kainate slices could be explained by early saturation of sigmoidal dependency, at a comparable level of maximal response.

Similar changes were observed in pEPSP slope curve plotted against FV. Additionally, the maximal amplitude of postsynaptic response in kainate group was twice higher than in control (Table 3). Thus, the fiber excitability and synaptic strength in CA3-CA1 connections markedly increased in kainate animals.

Effects of WIN55,212-2 and AM251 on the evoked activity in the hippocampus

The effect of cannabinoid compounds was estimated by comparison of I-O curves before and after their application. The effect of WIN55,212-2 and AM251 on the rate of “saturation” of responses (achievement of the plateau) was evaluated. CB receptor activation or inhibition did not change the I-O dependency of FV in control slices (Fig. 2B). After the application of WIN55,212-2 to kainate slices, the curve more rapidly reaches the plateau, due to significant increase of curve slope and a decrease of maximal amplitude parameters (p<0.05, compared to background) (Fig. 2B,C).

Conversely, after the application of AM251, both the I-O curves for FV and pEPSP slope “straightened” in control slices, indicating a linear dependence on the stimulation intensity (Fig. 2B,C). Thus, in this range of intensities, the saturation of population responses did not occur, indicating an increase in excitation in this communication system (Fleck et al. 2000; Norris, Scheff, 2009). It is interesting that after the application of AM251, the pEPSP slope dependency on the stimulation intensity in kainate group did not differ from the control despite significant distinctions in the FV I-O curve.

Figure 2D shows that the maximum excitement in the SC-CA1 system in kainate slices was not reached. However, it should be noted that the application of cannabinoid drugs did not substantially affect
the differences in the excitability in the SC–CA1 communication system (Table 3).

Discussion

Changes in the morphological state of the dorsal hippocampus after SE induced by KA

The study revealed neurodegeneration in the hippocampus one month after KA-induced SE. The most intensive death of neurons occurred in the hilus of dentate gyrus (field CA4) and, to a lesser extent, in the CA3a field of the dorsal hippocampus. Similar morphological changes after the intraventricular administration of KA at the same dose were reported by other authors (Gordon et al., 2014). In addition, consequences of degeneration after SE (10 days after the injection of KA), such as a decrease in the volume of the hippocampus and the dilation of ventricles, were revealed by life-time investigations in rats (Wolf et al., 2002). The extensive neurodegeneration in the hippocampus as a remote consequence of prolonged acute seizures bears witness to epileptogenesis and the emergence of a seizure focus (Morimoto et al., 2004). Thus, the intracerebral injection of kainate in our experiments led, as we believe, to the development of experimental epilepsy.

Changes in the activity of septal neurons

In our electrophysiological experiments on MS slices taken from the brain of healthy rats, the application of the antagonist of CB1 receptors AM251 led to a significant increase in the average frequency of cell discharges, i.e., in cell excitability. Conversely, the cannabinoid agonist WIN55,212-2 caused a decrease in the average neuronal rate, which, although insignificant, revealed a marked tendency to decrease neuronal excitability. It is evident that these changes result from the direct effect of the drugs on cannabinoid receptors present in the MS (Herkenham et al., 1991; Mailleux, Vanderhaeghen, 1992; Matsuda et al., 1993; Marsicano, Lutz, 1999). It was found earlier that one third of all cholinergic septal neurons express CB1 receptors (Nyiri et al., 2005); more than half of them express also GABAB receptors. Moreover, the coexpression of CB1, glutamatergic, and GABAergic markers was found in the MS (Hrabovszky et al., 2012). Thus, CB1 receptors are present in all three neurochemically different groups of neurons. Consequently, the activation or the blockade of these receptors can affect the release of acetylcholine,
glutamate, and GABA. There is substantial evidence for the existence of local cholinergic (Leranth, Frotscher, 1989; Gao et al., 1995; Brauer et al., 1998; Henderson et al., 2001; Mufson et al., 2003), glutamatergic (Wu et al., 2003), and GABAergic networks in MS (Henderson et al., 2001, 2004). Taking all possible intraseptal neuronal interactions into account, it is difficult to determine the mechanisms of changes in the average cell rate. The enhancement of discharging (and hence of excitability) after the application of AM251 may be due to the fact that the blockade of CB1 receptors on cholinergic and glutamatergic neurons leads to an increased release of acetylcholine and glutamate, which activate septal cells. The decrease in discharging after the application of WIN55,212-2 may result from the diminished release of exciting neurotransmitters. In all these cases, the changes in discharge frequency occur, presumably, only in a part of neurons of the corresponding neurochemical nature; however, it is these neurons that may be responsible for the total increase and decrease in excitability in the septal network.

In experiments on kainate slices of the MS, we observed a complicated pattern of changes in neuronal activity. The initial rate of cell discharges was substantially higher than that in control rats. A similar (almost twofold) increase in the average neuronal rate has been earlier observed in slices from the brains of guinea pigs with a model of TLE (Mal’kov et al., 2008). The experiments on the whole brain also revealed an increase in the neuronal activity in the MS of the epileptic brain (Colom et al., 2006; Kitchigina, Butuzova 2009). This indicates that the control of neuronal excitation in the MS in epileptogenesis is impaired, which is probably caused by the death/destruction of GABAergic septal neurons (Garrido-Sanabria et al., 2006). In the present work, it was shown for the first time that the frequency of cell discharges in MS kainate slices after the application of WIN55,212-2 significantly decreased and approached the activity in slices from healthy rats. This “normalization” of the activity in “epileptic” slices can be explained on the assumption that, upon the activation of CB1 receptors on cholinergic and glutamatergic neurons, as well as in “normal” slices, the release of exciting transmitters and hence their activating effect on MS neurons decreases by the action of WIN55,212-2. However, as opposed to experiments on brain slices from healthy animals, the CB1 receptor antagonist AM251 induced a nearly the same decrease in the frequency of discharges, which seems, at first glance, to be paradoxical. So far, we cannot explain these unidirectional effects of the agonist and antagonist of CB1 receptors. Most probably, they can result from changes in the receptor composition of MS cells in epileptogenesis and, as a consequence, the disturbance of neuronal interactions. Earlier Malkov and Popova (2011) showed an abrupt increase in the efficacy of the influence of GABA on rhythmic neurons of MS in an epileptic brain. The authors proposed that this effect is due to compensatory changes in GABA receptors, namely, an increase in their density and/or affinity in response to the death of a part of GABAergic neurons of MS. This assumption is supported by changes in the ratio of GABA subunits of receptors in MS cells in animals with a model of PTZ kindling (Follesa et al., 1999), which can change the sensitivity of these receptors. In our experiments, the blockade of CB1 receptors in GABAergic cells and an enhanced release of GABA led probably to a considerably stronger inhibition of neurons as compared with the normal brain, due to an increase in the sensitivity of GABA receptors. In addition, the enhancement of the inhibitory effect of GABA on cholinergic and glutamatergic neurons through the local network (Henderson et al., 2001, 2004) led probably to a diminishing in their excitatory action and a shift of the excitation/inhibition balance toward inhibition. All this can partly explain the decrease in the neuronal activity in “epileptic” slices after the application of AM251 instead of the increase in the control. Additional experiments are needed to confirm these assumptions.

The evidence available in the literature indicates that both natural and synthetic cannabinoids induce a long-term decrease in the power of both EEG and LFP, as well as in the neuronal activity in different brain structures (Buonomici et al., 1982; Ilan et al., 2004; Robbe et al., 2006; Robbe, Buzsaki, 2009; Hart et al., 2010; Goonawardena et al., 2011). The decrease in the frequency of discharges in the MS of healthy animals by the action of WIN55,212-2 in our experiments may be masked by a simultaneous decrease in the release of both inhibitory and excitatory neurotransmitters in neuronal networks of the MS by activated CB1 receptors. At the same time, in experiments on MS “epileptic” slices, the inhibitory effect of the CB1 agonist manifests itself more clearly, probably, due to the death of GABAergic cells (Colom et al., 2006) and
the preservation of the populations of cholinergic and glutamatergic MS neurons.

Changes in the evoked activity in the hippocampus

Our experiments revealed increase in excitability of SC in kainate slices: we showed higher average fiber volley to the stimulus with lower intensity. In addition, the maximal values of pEPSP slope evoked by SC stimulation in kainate slices were twice as high as in control animals, indicating a stronger excitability of hippocampal neurons in rats injected with kainate compared with healthy animals. One possible reason for the increase in the excitability in the hippocampus is the death of mossy cells of the hilus. It is known that glutamatergic mossy cells send their axons to interneurons of dentate fascia, which in turn form a GABAergic input to granular cells (Sloviter, 1991, Sloviter et al., 2003); the latter are projected onto pyramidal neurons of the CA3 field. The death of mossy cells results in the disinhibition of granular cells of dentate fascia and the enhancement of their excitatory input to the CA3 field. Because the dentate fascia (a part of dentate gyrus) contains neurons with unique properties, it was assumed to be the main hippocampal link that limits the excitatory input from the entorhinal cortex to the hippocampus (Hsu, 2007; Krook-Magnuson et al., 2015). In TLE models and in patients with TLE, the dentate fascia undergoes pathological changes, which can disturb its functions as a retarding factor and contribute to the development of TLE (Heinemann et al., 1992; Hsu, 2007). As shown in our morphological experiments, the strongest destructions occur just in the hilus of dentate

Figure 2. Effects of the cannabinoid agonist WIN55,212-2 and the CB1 receptor antagonist AM251 on the evoked electrical activity of the hippocampus one month after the intrabrain administration of normal saline (“Control”) or kainic acid (“Kainate”). (A) Typical pEPSP evoked by stimulation of Shaffer collaterals in hippocampal slices. (B, C) I-O curves for FV and pEPSP before (background) and after application of cannabinoid compounds. Sharp increase of responses in kainate slices is noticeable.
gyrus where mossy cells are localized and in the CA3 field of the hippocampus. These destructions usually result in the formation of free synaptic loci and the emergence of aberrant excitatory links (McNamara, 1994), which can lead to the enhancement of responses in the hippocampus observed in our study. The hypereexcitability in field CA3 was earlier shown on various experimental models of TLE, both kainic (Bragin et al., 1999; Wu, Leung, 2003) and pilocarpinic (Cossart et al., 2001).

In the experiments on hippocampal slices, we did not reveal any substantial effects of cannabinoid receptor agonist on the responses to SC stimulation. The effects were not observed in slices from the brain of both healthy and epileptic rats despite a substantially greater neuronal excitability in the animals of the second group. Despite the modulation of presynaptic responses (FV) by WIN55,212-2 in kainate slices, there was no significant difference in pEPSP I-O curve before and after application of the drug. The results of our experiments on slices of the normal brain are consistent with the results of other works where WIN55,212-2 (Paton et al., 1998) and 2-AG (Stella et al., 1997) also did not affect pEPSP. In addition, a low sensitivity of depolarization-induced pEPSP to WIN55,212-2 was revealed (Ohno-Shosaku et al., 2002). However, other authors observed a suppression of pEPSP in hippocampal slices (Ameri et al., 1999; Misner, Sullivan, 1999; Ameri, Simmet, 2000) and cell cultures (Shen et al., 1996; Sullivan, 1999) by the action of WIN55,212-2. These discrepancies may be due to methodical differences or the difference in the age of experimental animals. It was shown earlier that the effect of the agonist in the hippocampus depends on the target of its action; thus, on the separate stimulation of two inputs to CA3 formed by associative/comissural and mossy fibers, WIN55,212-2 inhibited the responses in CA3 to the stimulation of the first input and did not change the responses to the stimulation of the second input (Hofmann et al., 2008).

Application of AM251 caused the “straightening” of sigmoidal dependency in control slices, in such a way that resulting pEPSP I-O curve was not significantly differ from that in kainate group.

The data concerning the effect of the CB1 receptor antagonists on the activity of neurons are contradictory. Thus, it was found by some authors that the pharmacological blockade of cannabinoid receptors or their genetic elimination can facilitate the seizure activity or even induce it (Braakman et al., 2009; Marsicano et al., 2003; van Rijn et al., 2011) whereas the results of other investigations indicate that the antagonists of CB1 receptors do not substantially affect the LFP and the neuronal activity of the hippocampus (Coomber et al., 2008; Karr et al., 2010; Maier et al., 2012; Robbe et al., 2006).

It is also worth noting that during the registration of the neuronal activity in the hippocampus in vivo, no changes in the frequency of discharges of single neurons of the CA1 field by the action of the natural (Δ-9-tetrahydrocannabinol) and synthetic (CP55940) agonists of CB1 receptors were observed; however, the temporal coordination of the functioning of neurons was impaired; i.e., their activity was desynchronized (Robbe et al., 2006).

In our study, the analysis of evoked responses to SC stimulation revealed opposite changes in these responses, although insignificant, after the application of WIN55,212-2 and AM251, namely, a decrease and an increase, correspondingly. Thus, in both the hippocampus and the MS of the normal and epileptic brain, opposite changes in the excitability by the action of cannabinoid drugs were observed: a decrease in excitability in response to WIN55.212-2 and its increase by the action of AM251.

Some investigators observed a region-specific redistribution of hippocampal CB1 receptors during epileptogenesis (Falenski et al., 2007; 2009; Karlócai et al., 2011; Maglóczky et al., 2010), which could be the reason of the enhancement of GABAergic transmission and the reduction of the glutamatergic one. Taking these observations into account, it can be assumed that, in our

### Table 3. The effects of cannabinoid drugs on the evoked activity in the hippocampus one month after the intrabrain administration of normal saline (“Control”) or kainic acid (“Kainate”). I-O curve coefficients for fiber volley versus stimulus intensity, and pEPSP slope versus fiber volley are presented. Explanations in the text.

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<th>Control</th>
<th>Kainate</th>
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<td>Fiber volley vs stimulus intensity</td>
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<tr>
<td>A&lt;sub&gt;max&lt;/sub&gt;, mV</td>
<td>3.20±0.66</td>
<td>2.94±0.93</td>
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<tr>
<td>X&lt;sub&gt;0&lt;/sub&gt; (1/2 max stim), μA</td>
<td>582.2±58.8</td>
<td>33.0±23.3**</td>
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<td>b (curve slope), mV/μA</td>
<td>205.3±16.7</td>
<td>101.4±4.9*</td>
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<td></td>
<td>WIN55,212-2</td>
<td>WIN55,212-2</td>
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<td>AM251</td>
<td>1.03±2.15</td>
<td>1.60±0.81*</td>
</tr>
<tr>
<td></td>
<td>AM251</td>
<td>WIN55,212-2</td>
</tr>
<tr>
<td></td>
<td>2.98±1.37</td>
<td>52.0±16.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>253.0±75.3</td>
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<tr>
<td>EPSP slope vs fiber volley</td>
<td></td>
<td></td>
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<tr>
<td>A&lt;sub&gt;max&lt;/sub&gt;, mV/ms</td>
<td>3.97±0.74</td>
<td>7.63±1.20*</td>
</tr>
<tr>
<td>X&lt;sub&gt;0&lt;/sub&gt; (1/2 max stim), mV</td>
<td>0.30±0.02</td>
<td>0.43±0.02*</td>
</tr>
<tr>
<td>b (curve slope), 1/s</td>
<td>0.12±0.03</td>
<td>0.08±0.02*</td>
</tr>
<tr>
<td></td>
<td>WIN55,212-2</td>
<td>WIN55,212-2</td>
</tr>
<tr>
<td>AM251</td>
<td>3.31±0.72</td>
<td>11.64±2.85**</td>
</tr>
<tr>
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<td>0.35±0.06</td>
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<tr>
<td>AM251</td>
<td>13.67±1.49</td>
<td>0.42±0.25</td>
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<td>0.17±0.81</td>
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<tr>
<td></td>
<td></td>
<td>0.17±0.09*</td>
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<tr>
<td></td>
<td></td>
<td>0.15±0.03</td>
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</table>
| Data are presented as the mean±SEM * p<0.05, ** p<0.01 – significant difference between the “Kainate” and “Control” groups, Mann-Whitney U test
experiments, the changes in transmission in “kainate” hippocampal slices are the cause of the decrease and the increase in excitability in the SC–CA1 communication system under the influence of the cannabinoid agonist WIN55,212-2 and the CB1 receptor antagonist AM251, respectively.

Thus, our data demonstrate that both in the hippocampal and septal slices, the CB1 receptor antagonist AM251 led to a significant increase in cell excitability, and, on the contrary, the cannabinoid agonist WIN55,212-2 caused a decrease it. These results allow to assume protective impact of cannabinoid compound WIN55,212-2 on neuronal activity in the medial septum and hippocampus that disturbed by neurotoxic KA influence.

Conflict of interest statement
Nothing declared

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