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Cannabinoid receptor-1 activation suppresses inhibitory synaptic activity in human dentate gyrus☆

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Abstract

In spite of their popular uses as recreational drugs and their potential therapeutic uses, little direct information has been obtained about the synaptic effects of cannabinoids in the human brain. In the present study, patch-clamp recordings were performed on granule cells of the human dentate gyrus and the effects of cannabinoid receptor-1 (CB1) activation on inhibitory synaptic activity were examined. Activation of CB1 receptors by WIN55212-2 significantl suppressed both frequency and amplitude of spontaneous inhibitory synaptic currents (IPSCs) to about 50% of control. The suppressive effects were completely abolished in the presence of the CB1 receptor antagonist, AM251. WIN55212-2 also suppressed evoked IPSCs. However, neither frequency nor amplitude of miniature IPSCs were affected by WIN55212-2. These results provide electrophysiological evidence for the role of CB1 receptors in modulating inhibitory activity in human dent ate gyrus.

Keywords: Hippocampus; IPSCs; Human tissue; Dentate granule cell

1. Introduction

Cannabinoids, such as marijuana, are the most popular recreational drugs used worldwide. The psychological and neurological effects of cannabinoids in humans have been acknowledged for centuries. Recently, there has been increasing interest in the use of cannabinoids in many disorders including neurodegenerative diseases, pain and cancer (Smith, 2002; Bifulco and Di Marzo, 2002). Because of their profound psychological and neurological effects, clearly understanding how cannabinoids affect synaptic transmission in human brain may have important clinical implications both for therapeutic uses and for understanding side effects of cannabinoids.

Most psychological and neurological effects of cannabinoids are mediated by cannabinoid receptor-l (CB1), a G-protein-coupled receptor (Caulfiel and Brown, 1992; Matsuda et al., 1990). In addition to naturally occurring cannabinoids from plants, two endogenous cannabinoids, anandamide and 2-AG have been identifie in animals (Bisogno et al., 1999; Devane et al., 1992; Di Marzo et al., 1994; Stella et al., 1997). They can be released from neurons and mediate retrograde signaling at synapses in the brain including the hippocampus (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). The cannabinergic system may play an important physiological role in learning, memory and other brain functions. On the other hand, long-term exposure to exogenous cannabinoids such as marijuana may disturb physiological functions of the cannabinergic system and impair memory formation and learning ability. For example, marijuana impairs memory in laboratory animals. The impairment is so pronounced that the animals behave in some learning tasks as if the hippocampus had completely been removed (Heyser et al., 1993; Miller and Drew, 1973). Memory impairment has been associated with acute and chronic cannabis use in

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humans as well (Breivogel and Childers, 1998; Fletcher et al., 1996; Heishman et al., 1997; Stracciari et al., 1999).

The synaptic mechanisms by which cannabinoids affect neuronal activity are entirely extrapolated from electrophysiological studies performed in animals, mostly in rats. This is because fresh human brain samples that can be used for electrophysiological studies are not commonly available. Animal studies have shown that CB1 receptors are expressed mainly by GABAergic inhibitory interneurons in the hippocampus (Hoffman and Lupica, 2000; Katona et al., 1999; Tsou et al., 1999). The CB1-expressing interneurons are thought to control hippocampal oscillations (Freund and Buzsaki, 1996; Hajos et al., 2000). Through activation of CB1 receptors on GABAergic neurons, endogenous cannabinoids may modulate the synchronous spiking of hippocampal cells (Cobb et al., 1995). However, with the increasing body of evidence of species-related differences in the pharmacological profile and function of many receptors, it is necessary to verify those previous findings in animals with studies in human tissue when clinical relevance is taken into consideration. In the present study, human hippocampal specimens were obtained from patients as a part of surgical therapy for intractable temporal lobe epilepsy. Patch-clamp techniques were applied to neurons in the dentate gyrus to study the role of CB1-receptors in regulating inhibitory synaptic activity.

2. Methods

2.1. Tissue preparations

Temporal lobe epilepsy is the most common form of epilepsy that cannot be controlled with anticonvulsant medications. Many of these patients are treated surgically with anterior temporal lobectomy; a procedure that includes removal of the hippocampus and parahippocampal gyrus. In this study, human hippocampal specimens were obtained during anterior temporal lobectomies that were performed to treat intractable temporal lobe epilepsy in five patients (three males and two females) aged 9–56 years. All surgical specimens were evaluated by a neuropathologist. One patient had isolated hippocampal sclerosis. Two patients had benign tumors of the medial temporal lobe and associated hippocampal sclerosis. Two patients had no pathological changes. Tissue procurement and handling were performed as previously described (Murray et al., 2000; Zhu and Roper, 2001). In brief, specimens were obtained from the middle third of the hippocampus along its longitudinal axis, placed immediately in oxygenated Krebs solution at 4 °C, and taken directly to the laboratory for processing. The Krebs solution contained (in mM): NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11, pre-equilibrated with 95% O_2 and 5% CO_2. All procedures were approved by the Institutional Review Board of Shands Hospital at the University of Florida.

A block of hippocampal specimen from each patient was cut into five to ten sections (300 μm thick) using a Vibrotome (Technical Products International, St. Louis, MO). Slices were maintained in Krebs solution at room temperature and oxygenated continuously with 95% O_2 and 5% CO_2. Healthy neurons in slices can still be found even after 8 h in the above conditions. In each experiment, a hippocampal slice was transferred to a submerged recording chamber (*ca* 0.5 ml). A platinum grid was placed on the top of the slice to prevent slice movement. The slice was superfused (10 ml/min) with Krebs solution equilibrated with 95% O_2 and 5% CO_2 and maintained at room temperature (22 °C). Individual neurons were identified using an upright infrared, differential interference contrast (IR-DIC) microscope (IX70, Olympus America Inc., Melville, NY) with 40× magnification (0.8 NA objective).

2.2. Electrophysiology recordings

Whole cell patch-clamp recordings were made from neurons in dentate gyrus with electrodes (3–5 MΩ) filled with a solution containing (in mM): K^+-gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5. Neurons with membrane potentials in the range of –65 to -75 mV were considered to be healthy cells and were used for the experiments. To record inhibitory synaptic currents, cells were voltage-clamped at –10 mV. Signals were amplified and filtered at 2 kHz (Axopatch 200B) and sampled at 5 kHz. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the absence of tetrodotoxin (TTX) and miniature IPSCs (mIPSCs) recorded in the presence of TTX (0.5 μM, Sigma Chemical Co., St. Louis, MO). GABAergic mIPSCs were confirmed with the $GABA_A$ receptor antagonist bicuculline (20 μM, Sigma Chemical Co.). Stimulus-evoked IPSCs (eIPSCs) were recorded in bath solution containing 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM, Tocris Cookson, Ballwin, MO) and DL-2-amino-5-phosphonopentanoic acid (APV, 50 μM, Tocris Cookson) to block glutamatergic transmission. eIPSCs were elicited by focal stimulation of tissues near the recorded neurons (a glass electrode filled with Krebs solution, 3–4 MΩ). Cannabinoid receptor agonists (WIN55212-2, Tocris Cookson), antagonists (AM251, Tocris Cookson), and other testing compounds were applied through the bath solution. WIN55212-2 and AM251 were both prepared as 1 mM stock solutions dissolved in DMSO. The final concentration of DMSO in the bath solution was $<0.1\%$. Analysis of mIPSCs was performed as previously described (Gu and MacDermott, 1997) using the commercial software MiniAnalysis (Synaptosoft, Leonia, NJ,

www.jaejin.com). Data is presented as mean ± SEM. Student's *t* test was used for statistical comparison and signif cance was set at $p<0.05$.

3. Results

Whole-cell recordings were performed on granule cells in dentate gyrus (DGCs) of human hippocampal slices. DGCs could be identif ed using IR-DIC microscopy with a 40× objective (Fig. 1A and B) and further conf rmed on the basis of the action potential (AP) f ring pattern to depolarizing current injection (Fig. 1C). In these granule cells, a prolonged depolarization produced adaptation of AP frequency. Fig. 1C shows a typical example. Occasionally, we recorded neurons showing high frequency APs with little adaptation to the depolarization (Fig. 1D), consistent with the electrophysiological property of GABAergic interneurons. These putative interneurons were excluded from this study.

mIPSCs were recorded under voltage-clamp conf guration and in the presence of 0.5 µM TTX, 10 µM CNQX and 50 µM APV (Fig. 1E). mIPSCs showed outward or inward currents depending on holding potentials, the reversal potential was *ca* –60 mV (Fig. 1E and F). We used a holding potential of –10 mV for all the remaining experiments. The decay phase of mIPSCs could be well f tted into a two-exponential equation (Fig. 1G). The amplitude of mIPSCs showed a skewed distribution (Fig. 1H).

We f rst examined whether CB1 activation affects mIPSCs. In agreement with previous studies in rat hippocampus (Hoffman and Lupica, 2000; Katona et al., 2000; but see Irving et al., 2000; Wilson and Nicoll, 2001), bath application of 1 µM WIN55212-2, a CB1 receptor agonist, did not produce any signif cant effects on mIPSC frequency (Fig. 2A–C) or amplitude (Fig. 2A,D). The mIPSC frequency was 1.44±0.17 Hz in control and 1.40±0.25 Hz (i.e. 96±7% of control, n = 4) following 2 min application of WIN55212-2. The mIPSC amplitude was 17.0±1.3 pA in control and 16.9±1.3 pA (i.e. 99±2.3 % of control, n = 4) following application of WIN55212-2.

Spontaneous IPSCs were recorded in the absence of TTX (Fig. 3). Bath solution also contained 10 µM CNQX and 50 µM APV to block the potential inf uence of glutamatergic inputs. Similar to mIPSCs, the decay phase of sIPSCs could be well f tted into a two-exponential equation (Fig. 3B). In contrast to mIPSCs, bath application of 1 µM WIN55212-2 substantially suppressed sIPSC frequency (Fig. 3C and D) and prolonged inter-event intervals (Fig. 3E). In these experiments, WIN55212-2 (1 µM) was bath applied for 1 min. The suppressive effects began just after the end of drug application. The maximal suppressive effects occurred *ca* 2 min after the end of drug application (Fig. 3D). The frequency of sIPSCs was 0.94±0.11 Hz in control and signif cantly decreased to 0.40±0.06 Hz (45±9% of control, n = 4, $p < 0.05$, Fig. 3G) following bath application of 1 µM WIN55212-2. WIN55212-2 also had strong effects on sIPSC amplitude. The sIPSC amplitude histogram of

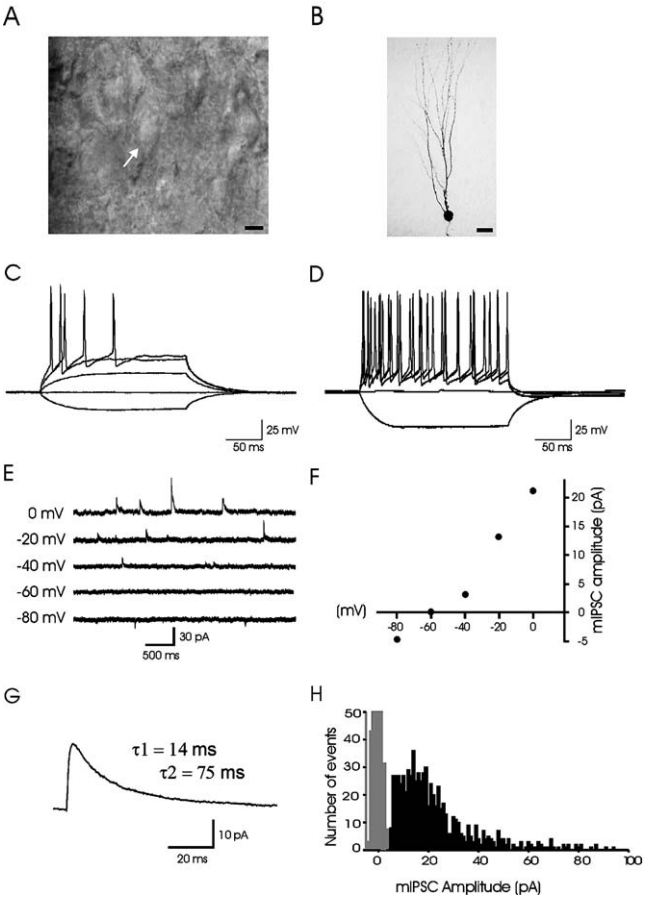

Fig. 1. Patch-clamp recordings from dentate gyrus of human brain. (A) Micrograph was taken from a human hippocampal slice, and the f eld was in the dentate gyrus area. Individual neurons were viewed under 40× IR-DIC microscopy for patch-clamp recordings. A neuron is indicated by an arrow in the f eld. Scale bar: 20 µm. (B) Image shows a biocytin-labeled granule cell in the dentate gyrus after a patch-clamp recording. Biocytin (0.1%) was included in the internal solution of recording electrodes. The dendrites extend into the molecular layer of the dentate gyrus (upper image) and the axon extends into the hilus (lower image). The soma appears more spherical than it actually was due to extravasation of a small amount of biocytin. Scale bar: 20 µm. (C) Membrane responses to a series of current injection in a neuron (–50–150 pA , 50 pA increments). The APs showed frequency adaptation. (D) Membrane responses to current injection in another neuron from the dentate gyrus. The f ring of APs showed little frequency adaptation. This f ring pattern is characteristic of an interneuron. (E) Sample traces show mIPSCs at different holding potentials recorded in a DGC. The bath solution contained 0.5 TTX, 20 µM CNQX, and 50 µM APV. (F) *I–V* relationship of mIPSCs showing a reversal potential of –60 mV. The averaged mIPSCs from the neuron shown in (E) were used for the *I–V* plot. (G) The trace shows an averaged mIPSC from 200 mIPSCs obtained at holding potential of –10 mV. The decay phase was f tted into a two-exponential equation, and the decay time constants are given in the f gure. (H) Histogram shows mIPSC amplitude distribution. The gray portion *ca* 0 pA represents noise distribution.

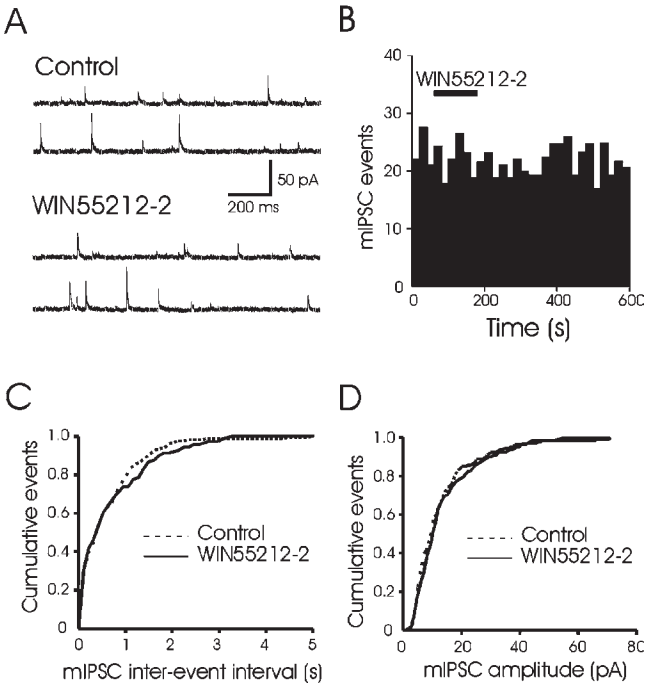

Fig. 2. The CB1 receptor agonist, WIN55212-2, has no effect on mIPSCs from human DGCs. (A) Sample traces of mIPSCs recorded from a neuron in normal bath solution (top two traces) and following the application of 1 μM WIN55212-2 (bottom two traces). (B) Time course of mIPSC frequency (number of events over time) for the experiment shown in (A). Each bin is 20 s. (C) The cumulative probability curve shows no change of inter-event intervals following the application of 1 μM WIN55212-2. (D) The cumulative probability curve shows no change of mIPSC amplitude following the application of 1 μM WIN55212-2. Similar results were obtained from three other DGCs. In all experiments, bath solution contained 0.5 μM TTX, 20 μM CNQX, and 50 μM APV; cells were held at –10 mV.

cumulative events showed a leftward shift, indicating a reduction in amplitude of sIPSCs (Fig. 3F). The average sIPSC amplitude was signif cantly decreased from 38±11 pA in control solution to 23±7 pA following bath application of 1 μM WIN55212-2 (i.e. 60±5% of control, n = 4, $p < 0.05$, Fig. 3G). The effects of WIN55212-2 were completely abolished in the presence of the CB1 receptor antagonist AM251 (Fig. 3G). sIPSC frequency was 98±3% of control and sIPSC amplitude was 100±5% of control (n = 3, $p > 0.05$) when 2 μM AM251 was present in bath solution during WIN55212-2 application (Fig. 3G). The effect of AM251 was due to its antagonism to the action of WIN55212-2 since AM251 itself did not have detectable effects on mIPSCs and sIPSCs (data not shown).

Under the same conditions as those used for recordings sIPSCs, eIPSCs could be elicited by focal stimulation near the recorded cells (Fig. 4A). The amplitude of eIPSCs was stable over time with continuous recording in normal bath solution (not shown). The amplitude of eIPSCs became substantially suppressed in the presence of 1 μM WIN55212-2. The amplitude of

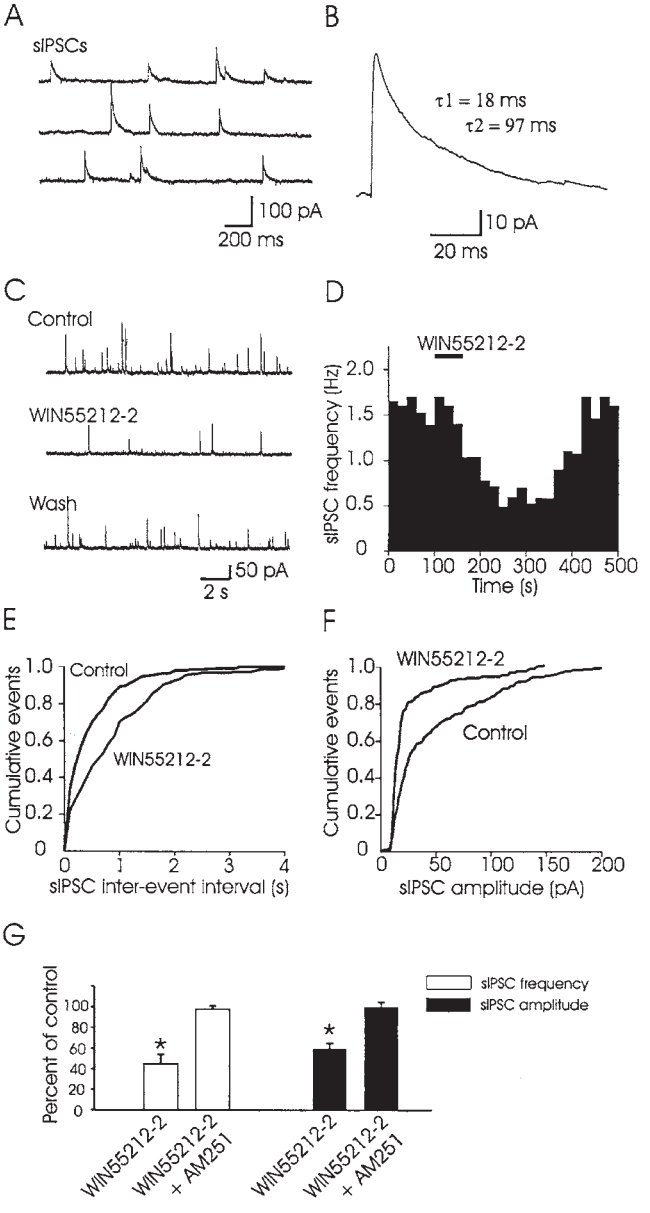

Fig. 3. CB1 receptor-mediated suppression of sIPSCs in the dentate gyrus of human brain. (A) Sample traces of sIPSCs recorded from a neuron in normal bath solution. (B) An averaged sIPSC from 200 sIPSCs. The decay phase was f tted into a two-exponential equation with the time constants given in the f gure. (C) Sample traces show sIPSCs before, following the application of 1 μM WIN55212-2, and after wash. (D) Time course shows the decrease of sIPSC frequency following the application of 1 μM WIN55212-2 for 1 min. (E) The cumulative probability curve shows an increase of inter-event intervals following the application of 1 μM WIN55212-2. (F) The cumulative probability curve shows a shift of sIPSCs to events with smaller amplitude following 1 μM WIN55212-2. (G) A summary of the changes in sIPSC frequency and amplitude by WIN55212-2 (n = 4) and the blocking effect by the CB1 receptor antagonist AM251 (2 μM) on WIN55212-2-induced responses (n = 3).

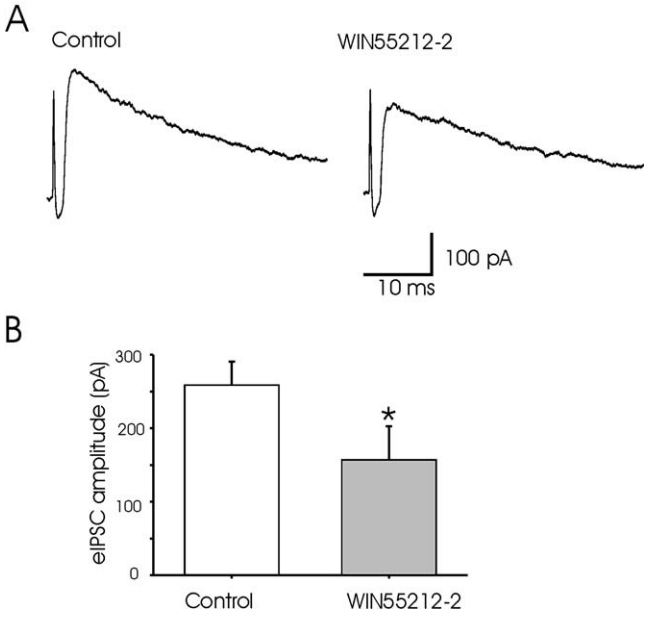

Fig. 4. CB1 receptor-mediated suppression of evoked IPSCs in the dentate gyrus of human brain. (A) Example shows an evoked IPSC from a representative DGC in normal bath solution (left) and suppression of eIPSCs following the application of 1 μM WIN55212-2 (right). (B) Summary of the experiments as shown in A (n = 9).

eIPSCs was 259±32 pA in control solution and it decreased to 175±46 pA in the presence of WIN55212-2 (65±3% control, n = 9, $p<0.05$, Fig. 4).

4. Discussion

To our knowledge, these data are the first to provide evidence that activation of CB1 receptors affects IPSCs in human brain and they complement a previous report that CB1 agonists attenuate stimulus-evoked release of [^{3}H]GABA from human hippocampal slices (Katona et al., 2000). Our results indicate that the GABAergic inhibitory system in dentate gyrus is an important target of exogenous and, perhaps, endogenous cannabinoids. We have shown that both sIPSCs and eIPSCs in dentate gyrus were suppressed following CB1 receptor activation. This is consistent with the effects of marijuana on learning, memory and cognition in human. Through the reduction of GABAergic inhibitory activity, exogenous cannabinoids may cause a non-specific increase in excitatory firing (noise) that could impair information processing in the hippocampus. Conversely, activation of CB1 receptors in human dentate gyrus by endogenous cannabinoids may play an appropriate role in learning and memory formation under physiological conditions.

While pharmacological profiles of many receptors show tissue and species differences, our results appear to be consistent with those of previous studies in rats (Davies et al., 2002). For example, activation of CB1 receptors by WIN55212-2 has also been shown to suppress frequency and amplitude of sIPSCs as well as the amplitude of the eIPSCs in rat hippocampus (Hajos et al., 2000; Hoffman and Lupica, 2000). We have found that neither mIPSC frequency nor mIPSC amplitude was affected by WIN55212-2 in human dentate gyrus. The lack of effect on mIPSC amplitude suggests that WIN55212-2 has little effect on postsynaptic $GABA_A$ receptors. Thus, the effects by WIN55212-2 on sIPSCs and eIPSCs are likely due to an action on presynaptic GABAergic neurons. This is consistent with immunohistochemical studies that show localization of CB1 receptors on hippocampal interneurons (Katona et al., 1999, 2000; Tsou et al., 1999).

Studies in rat hippocampal slices and cultured hippocampal cells have reported either suppression of mIPSC frequency (Irving et al., 2000; Wilson and Nicoll, 2001) or no effect on mIPSCs by CB1 receptor agonists (Hajos et al., 2000; Hoffman and Lupica, 2000). The reason for this discrepancy is currently unclear. The suppression of mIPSC frequency provides correlative physiological evidence that CB1 receptors are expressed at the presynaptic terminals of GABAergic neurons. However, the lack of effect on mIPSC frequency in the present study does not necessarily mean the lack of CB1 receptors at the presynaptic terminals of GABAergic neurons in human dentate gyrus. CB1 receptors at presynaptic terminals in human dentate gyrus may be not directly coupled with release machinery so that mIPSCs are not affected during CB1 activation. It has been shown that activation of CB1 receptors decreases the activity of voltage-gated Ca^{2+} channels. This action, if occurs at presynaptic terminals, would suppress sIPSCs and eIPSCs without affecting mIPSCs. Alternatively, the primary action of CB1 receptors may be to decrease excitability of interneurons; another scenario where sIPSCs and eIPSCs could be affected without altering mIPSCs.

In our study, dentate gyrus specimens of human hippocampus were obtained during surgical procedures to treat intractable medial temporal lobe epilepsy. Hippocampi from people with epilepsy may be abnormal due to effects of the epileptogenic process as well as long-term exposure to drugs that are used to treat seizures. Therefore, we cannot be certain whether our results represent normal or pathological functions of the human hippocampus. Since these responses were seen from patients with and without hippocampal sclerosis (the most common pathological substrate of temporal lobe epilepsy); our results do not appear to be a unique property of this particular pathological process. Interestingly, cannabinoids may have both anti-epileptic and pro-epileptic effects in patients depending upon the type of epilepsy in question (Gordon and Devinsky, 2001). This raises an interesting issue whether effects of cannabinoids on inhibitory synaptic activity are different in different types of epilepsy patients. This study confirms that CB1 receptors are powerful modulators of inhibitory

synaptic activity in human dentate gyrus and, as such, may play important roles in normal hippocampal function and pathological states.

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