Involvement of the Endocannabinoid System in Ethanol-Induced Corticostriatal Synaptic Depression

Hyeong Seok Cho1,2, Seung Hyun Jeun1,2, Qing-Zhong Li1,2, Ki Jung Kim1,2, Se Joon Choi1,2, and Ki-Wug Sung1,2,*

1Department of Pharmacology, 2MRC for Cell Death Disease Research Center, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Korea

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Abstract. Ethanol is a wildly abused substance that causes various problems and damage in our society. We examined the connection between the action of ethanol and the endocannabinoid system in corticostriatal synaptic transmission by recording excitatory post-synaptic currents (EPSCs). Acute treatment of ethanol (100 mM) inhibited corticostriatal EPSCs. In the presence of AM 251 (5 μM), a cannabinoid 1 (CB1)-receptor antagonist, or AM 404 (5 μM), a cannabinoid transporter inhibitor, the inhibition of corticostriatal EPSCs caused by ethanol was significantly reduced. This result suggests the possibility that the endocannabinoid system is involved in the action of ethanol. To support this result, brain slices were pre-treated with WIN 55,212-2 (1 μM), a CB1-receptor agonist, following treatment of ethanol or treated with WIN 55,212-2 alone. There was no significant difference between each other, indicating that when CB1 receptors are previously activated, the effect of ethanol is blunted. These results suggest that the activation of the endocannabinoid system is one of the possible mechanisms involved in ethanol-induced corticostriatal synaptic depression.

Keywords: striatum, endocannabinoid, ethanol, synaptic depression

Introduction

The dorsal striatum is the major input nuclei in the basal ganglia. It receives glutamatergic inputs from the cortex and the thalamus, and these synaptic transmissions play an important role in the generation of voluntary movement (1, 2). The dorsal striatum also plays critical roles in learning and memory. Recent studies reported that the dorsal striatum has an important role in skill, instrumental, and response-based learning (3 – 6). In addition, the dorsal striatum is a target of drug abuse and addiction, including compulsive alcohol or drug taking and seeking behaviors (7).

The cannabinoid 1 (CB1) receptors are widely expressed in the brain with specific neuronal locations in areas related to cognitive processes (neocortex and hippocampus), motor control (basal ganglia and cerebellum), and emotional response (amygdala) (8). The CB1 receptors are abundant in these brain regions and play an important role in the regulation of the reward circuits commonly activated by abused drugs including ethanol (9). Also, the endocannabinoid system has an essential role in modulating the synaptic activity of the dorsal striatum. Little is known about the contributions of striatal synaptic plasticity to learning and memory, but many researchers reported that the endocannabinoid system could modulate corticostriatal synaptic activity and is a key factor in corticostriatal long-term depression (LTD) (10 – 13).

From behavioral tests and studies of other brain regions, the possibility that there is a connection between the action of ethanol and the endocannabinoid system in the dorsal striatum arises. When the endocannabinoid system was interrupted, behavioral symptoms that are related to alcoholism were suppressed (14 – 17). In addition, studies from the hippocampus and the amygdala reported that the endocannabinoid system modulates the effect of ethanol on synaptic transmission (18, 19); and from our previous study, we reported that ethanol inhibits the corticostriatal synaptic transmission in a dose-depen-
dent manner (20). Taking these reports together, we hypothesized that the endocannabinoid system may be involved in ethanol-induced corticostriatal synaptic depression. Therefore, we tested the effects of cannabinoid drugs on synaptic depression induced by ethanol in rat corticostriatal brain slices.

Materials and Methods

Slice preparation

Brain slices were prepared from 14 to 20-day-old Sprague-Dawley rats using previously described techniques (21). Rats were anesthetized with CO₂ and sacrificed by decapitation in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Brains were removed and placed in ice-cold, modified artificial cerebrospinal fluid (aCSF) containing 194 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 10 mM D-glucose, adjusted to pH 7.4 by bubbling with 95% O₂ / 5% CO₂. Coronal slices (300-μm-thick) were cut using a vibrotome (Campden Instruments, Loughborough, UK). Brain slices were transferred to aCSF containing 124 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 10 mM D-glucose, adjusted to pH 7.4 by bubbling with 95% O₂ / 5% CO₂ at room temperature. Slices were used for experiments 2 h after the slice preparation. A hemislice containing the cortex and striatum was completely submerged in a recording chamber and continuously superfused with aCSF that was constantly bubbled with 95% O₂ / 5% CO₂. The flow rate was kept at 2 – 3 ml/min using a peristaltic pump (Glison, Villiers Le Bel, France). The temperature of the bath solution was maintained at 30°C – 32°C.

Whole-cell recording

Whole-cell voltage-clamp recordings were performed to record excitatory post-synaptic currents (EPSCs) at the striatal synapses (21). Electrical stimuli (0.05 Hz, 0.1 ms, 0.5 – 1 mA) were delivered through a bipolar, Teflon®-coated tungsten electrode placed in the white matter dorsal to the striatum and close to the recording electrode. The amplitude of baseline EPSCs were 50 – 100 pA. Whole-cell recordings were obtained using pipettes made from borosilicate glass capillaries pulled by a P-97 micropipette puller (Sutter Instruments, Novato, CA, USA). Pipettes (3 – 5 MΩ) were filled with internal solution containing 120 mM CsMeSO₃, 5 mM NaCl, 10 mM tetraethylammonium chloride, 10 mM HEPES, 5 mM QX-314, 1.1 mM EGTA, 4 mM Mg-ATP, 0.3 mM Na-GTP, pH adjusted to 7.2 with CsOH and osmolarity adjusted to 290 – 300 mOsm with sucrose. Medium-sized neurons within two or three layers below the surface of the slice were identified using an Olympus BX50WI (Olympus, Tokyo) differential interference contrast microscope. Neurons were voltage-clamped at −70 mV, and EPSCs recorded with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) were filtered at 5 kHz, digitized at 10 kHz using a Digidata 1322A (Molecular Devices), and stored on a personal computer using pClamp 10 software (Molecular Devices). Series resistance ranged between 10 – 30 MΩ and was not compensated. When series resistance changed more than 20% during experiments, the data was excluded from analysis. All experiments were performed with paired-pulse stimulation, and the paired-pulse ratio (PPR) was calculated as the ratio of the amplitude of the second EPSC to the first EPSC with an interpulse interval of 50 ms.

Drugs and chemicals

AM 251, AM 404, and WIN 55,212-2 were purchased from Tocris Cookson (Avonmouth, UK). All other chemicals were purchased from Sigma (St. Louis, MO, USA). Drugs were diluted with aCSF before use from the stock solutions and were delivered to the recording chamber through a peristaltic pump. AM 251, AM 404, and WIN 55,212-2 were dissolved in DMSO and the final concentration of DMSO in ACSF was under 0.01%.

Data analyses

All averaged data were expressed as means ± S.E.M. The mean values 10 min prior to the drug treatment from each slice were averaged and defined as a baseline response and used as a control. All EPSCs data were normalized to the baseline response and the drug responses of both EPSC amplitude and PPR were compared to this value. The statistical significance of changes in synaptic responses was determined using Student’s t-test and one-way ANOVA with Newman-Keuls post test. The statistical criterion for significance was P < 0.05.

Results

Treatment with ethanol (100 mM) for 10 min significantly decreased corticostriatal EPSC amplitude (Fig. 1). EPSC amplitude decreased 32.3% ± 3.3% (P < 0.05, n = 15) from the baseline and there was a significant increase in the PPR (from 1.3 ± 0.1 to 1.6 ± 0.1, P < 0.05, n = 15), which suggests the inhibition is caused by the decrease of pre-synaptic glutamate release.

To examine the relationship between the endogenous cannabinoid system and ethanol-induced corticostriatal synaptic depression, we used AM 251 (5 μM), a CB₁-receptor antagonist, and AM 404 (5 μM), a cannabinoid
transporter inhibitor, to block the endocannabinoid system. When AM 251 and AM 404 were pre-applied, ethanol inhibited corticostriatal synaptic transmission by 14.4% ± 3.9% (n = 5) and −1.4% ± 8.7% (n = 7), respectively (Fig. 2: A, B). As shown in Fig. 1, ethanol alone inhibited corticostriatal synaptic transmission by 32.3% ± 3.3%, and compared to this data, the synaptic depression caused by ethanol was significantly smaller when AM 251 or AM 404 was pre-applied (P < 0.05, Fig. 2C). These results suggest that the effect of ethanol on corticostriatal synaptic transmission is modulated by the endocannabinoid system.

To support that these results are caused by the endocannabinoid system, brain slices were pre-treated with WIN 55,212-2 (1 μM), a CB1-receptor agonist, following ethanol treatment or treated with WIN 55,212-2 alone. We assumed that if ethanol mediates its action through the endocannabinoid system, there would be no difference in the inhibition caused by ethanol when the CB1 receptors were pre-activated. When ethanol was applied to the brain slice with WIN 55,212-2 pre-treatment, it decreased corticostriatal EPSCs 25.3% ± 12.0% (n = 13) from the baseline, and when WIN 55,212-2 was applied alone, it inhibited corticostriatal EPSCs 39.8% ± 6.8% (n = 14) from the baseline. The amount of inhibition caused by ethanol or WIN 55,212-2 or ethanol with WIN 55,212-2 pre-treatment showed no significant difference (Fig. 3), which indicates that they might all have the same pathway in common. Taken together, our results suggest that the endocannabinoid system seems to play a role in ethanol-induced depression of corticostriatal synaptic transmission.

**Discussion**

The endocannabinoid system modulates both the excitatory and inhibitory synaptic transmission of the dorsal striatum (11, 22), and the CB1 receptors show enriched expression in the striatum (8, 23). Our results showed that the acute treatment of ethanol depresses corticostriatal synaptic transmission, and this synaptic depression appears to involve the endocannabinoid system. In addi-
tion, there are several other reports, including behavioral studies, showing the connection between the endocannabinoid system and the action of ethanol in the striatum. Once the endocannabinoid system is blocked, behaviors such as self-administration, sensitivity, and dependence, which are known to be related to alcoholism, were suppressed (14–16).

The CB1 receptors are located at the pre-synaptic terminals and endocannabinoid is synthesized from the post-synaptic neurons and acts as a retrograde messenger. As shown in Fig. 1, when ethanol treatment was applied, there was a significant increase in the PPR, which suggests that ethanol has a pre-synaptic mechanism in its action on corticostriatal synaptic transmission. There is a discrepancy in the PPR data compared to our previous study, which showed no change of the PPR by ethanol (20). We decreased the stimulus protocol to get the baseline EPSCs to 50–100 pA to exclude the possibility of overstimulation by strong stimulus intensity. Also, despite the difference in brain region, a study of the hippocampal neurons also showed that acute ethanol treatment enhanced the formation of endocannabinoids (18), a result that supports our finding. Also, as shown in Fig. 1, EPSCs amplitude inhibited by ethanol did not show a full recovery, this could be caused by activation of the endocannabinoid pathway since CB1-receptor agonist can induce LTD (12). Altogether, the evidence suggests that the endocannabinoid system has an important role in the action of ethanol through various brain regions including the dorsal striatum. Furthermore, the endocannabinoid system plays a role in regulation of the reward circuits commonly activated by abused drugs including ethanol (24, 25). In addition, a recent study reported that impaired endocannabinoid signaling reduces alcohol intake, which suggests endocannabinoid signaling as a candidate mechanism in alcohol reward (26). Combining the results of behavioral and electrophysiological studies on ethanol and the endocannabinoid system provides clues for why the behavioral symptoms caused by alcohol abuse or addiction are suppressed when the endocannabinoid system is antagonized.

In conclusion, our results may contribute to the better understanding of the effect of ethanol on synaptic transmission in the dorsal striatum. However, the mechanism of ethanol’s action in triggering the endocannabinoid pathway in the striatum is still unknown. Further studies will be focused on examining specific targets of action of ethanol in the endocannabinoid system.

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References