Differential Sensitivity of GABA<sub>A</sub> Receptor-Mediated IPSCs to Cannabinoids in Hippocampal Slices From Adolescent and Adult Rats

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INTRODUCTION

The use of marijuana remains strikingly high among adolescents in the U.S. Among more than 2 million Americans who used marijuana for the first time in 1999, 2/3 of them were between the ages of 12 and 17 (DHHS 2002). This raises concern because one of the most salient cognitive effects of Δ<sub>9</sub>-tetrahydrocannabinol (THC, an active ingredient of marijuana), impairs spatial learning more efficaciously in adolescent rats, compared with adult rats, but there have been no studies of the cellular mechanisms underlying this developmental sensitivity. In this study, we examined cannabinoid-mediated activity in hippocampal area CA1 neurons in brain slices from adolescent and adult rats. The magnitude of endocannabinoid-mediated synaptic functions such as long-term depression of inhibition was greater in the hippocampal slices from adolescent rats than in those from adults. The effect of R-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazine-6-yl)(1-naphtalenyl) methanone mesylate (WIN55,212-2), an exogenous cannabinoid agonist, to suppress GABA<sub>A</sub> receptor-mediated synaptic responses was also greater in the hippocampal slices from adolescent rats than in those from adults. However, tonic endocannabinoid effects, shown as an increase of the spontaneous IPSC frequency by N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-di-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), a specific CB1 receptor antagonist, were greater in CA1 neurons from adult rats than in those from adolescent rats. On the other hand, WIN55,212-2 suppressed glutamate-mediated excitatory neurotransmission in CA1 pyramidal cells from adolescent and adult rats with similar efficacy. These results indicate that inhibitory synaptic function in the adolescent hippocampus is more sensitive to cannabinoid effects and may account, in part, for the greater sensitivity of adolescent animals to THC-induced memory impairment.
We hypothesized that the different sensitivity of adolescent and adult animals to the memory-impairing effects of THC could be due to developmental differences in the function of cannabinoid receptors in the hippocampus. In the present study, we have begun to address this hypothesis by assessing the effects of long-term depression of inhibition (I-LTD) as well as the effects of the cannabinoid agonist WIN55,212-2 on GABA<sub>A</sub> receptor-mediated inhibitory function and glutamate receptor-mediated excitatory function in hippocampal slices from adolescent and adult rats.

**Methods**

A total of 80 male Sprague-Dawley rats, adolescent (28–35 postnatal days, PD) and adult (75–110 PD), were purchased from Charles River Laboratories (Raleigh, NC) and housed in groups on a 12-h light-dark cycle with ad libitum access to food and water. All experimental procedures were conducted in accordance with Institutional Animal Care and Use Committee guidelines and were approved by the institution.

**Slice preparation and electrophysiology**

Brains were rapidly removed from rats under halothane anesthesia. Removed brains were immersed in ice-cold oxygenated (95% O<sub>2</sub>-5% CO<sub>2</sub>) modified artificial cerebrospinal fluid (ACSF). ACSF contained (in mM) 120 NaCl, 3.3 KCl, 1.23 Na<sub>2</sub>HPO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 2 CaCl<sub>2</sub>, 0.9 MgSO<sub>4</sub>, and 10 glucose, whereas modified ACSF has 0.5 CaCl<sub>2</sub> and 2 MgSO<sub>4</sub>. Coronal slices (400 μm) containing the hippocampus were cut using a Vibratome (Campden, model 752, England) and incubated in ACSF that was continuously bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> at room temperature. After minimum 1-h incubation, a single slice was transferred to the recording chamber (volume: 0.5 ml). Oxygenated ACSF was perfused over submerged slices at a rate of ~3–4 ml/min. Individual cells were viewed with an upright Axioskop fixed-stage microscope (Zeiss Axioskop, Thornwood, NY) equipped with a water-immersion objective (×40, 0.75 numerical aperture), IR filtered light, differential interference contrast (DIC) optics, and a Hitachi CCD camera (Tokyo, Japan). For recording, patch pipettes were pulled from borosilicate glass capillary tubing (1.5 mm OD, 1.05 mm ID, World Precision Instruments, Sarasota, FL) using a Flaming-Brown horizontal microscope electrode puller (model P-97, Sutter Instrument, Novato, CA). The pipettes (input resistance: 2–5 MΩ) were filled with the following recording solution (in mM): 100 CsCl, 60 Cs<sub>2</sub>SO<sub>4</sub>, 10 HEPS, 0.2 EGTA, 1 MgCl<sub>2</sub>, 4 Mg-ATP, 0.3 Tris-GTP, and 5 QX314 (pH 7.25, 285 mOsm). In some experiments, 140 mM CsCl was used instead of 100 mM CsCl and 60 mM Cs<sub>2</sub>SO<sub>4</sub> to enhance driving force for Cl<sup>-</sup> and 10 mM disodium creatine phosphate were added.

Whole cell patch recordings were made on CA1 pyramidal neurons. Cells were voltage-clamped at ~70 mV, and recordings were performed at room temperature. Series resistance (13–20 MΩ) was monitored on-line throughout the experiment using a digital oscilloscope (Nicolet model 410), and the cells were rejected if the resistance changed by >20%. No series resistance compensation was used. Synaptic responses were evoked using a monopolar tungsten electrode (A-M System, Carlsborg, WA). The stimulating electrode was placed in stratum radiatum close to the pyramidal layer. Stimuli were square-wave current pulses (0.05-ms duration) delivered every 30 s. The stimulus strength was adjusted to evoke 70% maximal amplitude as determined by the input/output relationship (Fig. 1). The range of the stimulus strength was 40–70 μA. GABA<sub>A</sub> receptor-mediated inhibitory post-synaptic currents (IPSCs) were pharmacologically isolated by adding the glutamate receptor antagonists, d-(-)-2-amino-5-phosphonovaleric acid (d-AP5, 50 μM) and 6,7-dinitroquininalo-xine-2,3-dione (DNQX, 20 μM), to the ACSF and were verified by the GABA<sub>A</sub> receptor agonist bicuculline methiodine (BMI, 20 μM; Fig. 1). Glutamate receptor-mediated fast excitatory post-synaptic currents [EPSCs, non-N-methyl-D-aspartate (NMDA) receptor mediated] were pharmacologically isolated by adding d-AP5 (50 μM) and BMI (20 μM) to the ACSF, and were verified by non-NMDA glutamate receptor antagonist, DNQX (20 μM; Fig. 1).

For LTD experiments, cells were voltage clamped at +10 mV. After 20 min of stable baseline, I-LTD was induced by theta burst stimulation (TBS) consisting of a series of 10 bursts of five stimuli (100 Hz within burst, 200-ms interburst interval), which was repeated four times at 5-s intervals. The magnitude of LTD was calculated by comparing averaged responses 20–25 min after induction to baseline-averaged responses before induction. In some case, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A, 1 μM), a CB1 receptor specific antagonist/inverse agonist, was applied 25 min before the induction of I-LTD.

For cannabinoid agonist experiments, WIN55,212-2 (0.1–5 μM) was applied for 10–15 min individually or sequentially, after a 10-min baseline-recording period. The cannabinoid CB1 receptor specific antagonist/inverse agonist, SR141716A, was applied 25 min before the induction of I-LTD.

**Results**

**Electrophysiological recordings**

**A. GABA<sub>A</sub> receptor-mediated inhibitory function and glutamate receptor-mediated excitatory function in hippocampal slices from adolescent and adult rats.**
antagonists, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251, 1 or 5 μM) was applied in the presence of WIN55,212-2 through separate tubing to avoid contamination and responses were observed for another 20–30 min.

Data acquisition and analysis
IPSCs and EPSCs were recorded using an Axopatch 200B (Axon Instruments, Foster City, CA), filtered at 2 kHz (-3dB), and digitized by Lab PC+ (National Instruments, Austin, TX). Evoked IPSCs and EPSCs were acquired at 10 kHz using LabView (National Instruments, Austin, TX) and analyzed off-line using LabView (National Instruments, Austin, TX) on an IBM-compatible computer. Results are taken from one slice from one animal in each condition. For the analysis, IPSC or EPSC peak amplitude was measured. The effect of WIN55,212-2 and the magnitude of I-LTD were tested using paired t-test. Results between groups were compared using t-test for independent samples. For experiments with multiple doses or multiple stimulus intensities, results were compared using one- or two-way ANOVA. Spontaneous IPSCs (sIPSCs) were measured using MiniAnalysis software (www.syanptomsoft.com). Statistical significance of the differences in the frequency and amplitude of sIPSCs before and after drug application were assessed using Kolmogolov-Smirnov tests. Comparisons between averaged responses from adolescent and adult CA1 neurons were made using independent Student’s t-test. For all statistical tests, a P value of <0.05 was set as the criterion for statistical significance. SPSS (SPSS, Chicago, IL) was used for all statistical analyses, and Origin (Origin Lab, Northampton, MA) was used for plotting all figures. All grouped data are represented as means ± SE.

Drugs
The drugs used for these experiments were BMI, DNQX, d-AP5, WIN55,212-2, AM251, and SR141716A. All drugs were purchased from Sigma (St. Louis, MO) except WIN55,212-2 and AM251, which were purchased from Tocris Cookson (Bristol, UK), and SR 141716A was a generous gift from National Institute of Drug Abuse Chemical Synthesis and Drug Supply Program.

Results
We made whole cell patch recordings from CA1 pyramidal neurons from adolescent (28–35 PD) and adult (75–110 PD) rats. There were no differences in the resting membrane potential or input resistance between the two age groups. The resting membrane potentials were -69.6 ± 0.6 mV (n = 15) for adolescent rats and -68.6 ± 0.3 mV (n = 33) for adult rats (P = 0.09 between groups) without compensation of the junction potential. The input resistances were 105.5 ± 5.3 MΩ (n = 15) and 99.4 ± 2.8 MΩ (n = 33) for adolescent and adult rats, respectively (P = 0.26 between groups). In addition, the input/output functions of excitatory and inhibitory synaptic responses were comparable between adolescent and adult rats. There was no difference in the EPSC or IPSC amplitudes across different stimulus intensities; EPSC amplitudes [F(1,46) = 0.03, P = 0.86] and IPSC amplitudes [F(1,41) = 1.06, P = 0.31] between adolescent and adult rats (Fig. 1).

First we assessed the function of endogenous cannabinoid systems in CA1 pyramidal neurons from adolescent and adult rats using I-LTD. I-LTD can be induced by the same stimulation protocols that are used to induce long-term potentiation of excitatory synapses, such as high-frequency stimulation or TBS. TBS induced I-LTD in slices from adolescent (30.0 ± 4.7% LTD; n = 6, P < 0.01) and adult (14.2 ± 4.2% LTD; n = 6, P = 0.04) rats (Fig. 2, A and B). We found that the magnitude of I-LTD was significantly greater in the hippocampal slices from adolescent rats compared with those from adult rats (P = 0.03 between groups comparison; Fig. 2, A and B). After 25-min application of SR141716, a CB1 antagonist, TBS failed to induce I-LTD (2.1 ± 5.4% LTD, n = 4, P = 0.69), confirming that I-LTD is mediated by CB1 activation (Fig. 2, A and B).

To address the direct effect of CB1 receptor stimulation in slices from animals of different ages, we assessed the effects of the exogenous CB1 receptor agonist, WIN55,212-2, on the inhibitory neurotransmission. WIN55,212-2 (1 μM) significantly decreased the amplitude of evoked IPSCs in CA1 pyramidal cells from adolescent and adult rats, and this effect was reversed by AM251, a specific CB1 receptor antagonist, confirming that the agonist effect was mediated through CB1 receptors (Fig. 3). The magnitude of the WIN55,212-2 effect on evoked IPSCs was significantly greater in neurons from adolescent rats than in those from adults (P = 0.02; Fig. 3). WIN55,212-2 decreased evoked IPSCs by 42.1 ± 3.7% (n = 6, P < 0.01) and 24.1 ± 5.3% (n = 6, P = 0.02) from

![FIG. 2. Long-term depression of inhibition (I-LTD) magnitude in the CA1 hippocampal area is greater in slices from adolescent rats than adult rats. Cells were voltage clamped to +10 mV and I-LTD induced by theta burst stimulation (TBS, arrowhead in A) in s. radiatum. A: average time-dependent responses from adolescent (○, n = 6) and adult (●, n = 6) rat hippocampal slices. Inset: representative traces before (1) and 20–23 min (2) after I-LTD induction from adolescent (a) and adult (b) rat hippocampal slices. Scale bars are 0.1 nA and 100 ms. ▲, I-LTD responses after 25-min application of SR141716A. B: average I-LTD magnitudes from adolescent (□) and adult (▲) rats, * P < 0.05. The I-LTD magnitude in adolescent rats (30.0 ± 4.7%, P < 0.01) is significantly greater than in adult rats (14.2 ± 4.2%, P = 0.04; P = 0.032 between groups). □, I-LTD magnitude in the presence of SR141716 (2.1 ± 5.4%, P = 0.69).](http://jn.physiology.org/10.1152/jn.02034.2016/download)
adolescent and adult rats, respectively. In addition, WIN55,212-2 increased the paired-pulse ratio, and this effect was reversed by AM 251, confirming the presynaptic mechanism of WIN55,212-2. In adolescent rats, the paired-pulse ratio was increased from 0.61 to 0.96 (62.6 ± 17.0%, P < 0.01), whereas the paired-pulse ratio was increased from 0.63 to 0.76 (20.4 ± 3.1%, P < 0.01) in the adult rats. The magnitude of the increase in paired-pulse ratio was significantly different between adolescent and adult rats (P = 0.03). We further examined the dose-responses of WIN55,212-2 to investigate the age difference in the maximal effect and EC50 of WIN55,212-2 between adolescents and adults (Fig. 3E). At the concentration of 0.1, 0.3, 1, 3, and 5 μM, WIN55,212-2 decreased the amplitudes of eIPSCs by 20.4 ± 2.7% (n = 12), 31.5 ± 2.9% (n = 18), 40.0 ± 3.1% (n = 15), 44.0 ± 2.4% (n = 15), and 44.0 ± 2.5% (n = 14), respectively, in CA1 neurons from adolescent rats. The same concentrations of WIN55,212-2 decreased the amplitude of eIPSCs by 11.8 ± 2.2% (n = 8), 22.5 ± 2.5% (n = 13), 30.0 ± 3.5% (n = 13), 37.0 ± 2.8% (n = 13), and 40.0 ± 3.2% (n = 12), respectively, in the CA1 hippocampal neurons from adult rats. At the submaximal doses such as 0.1, 0.3, 1 μM, WIN55,212-2 effects were significantly greater in the adolescent rats than adult rats with F(1,18) = 5.4 (P = 0.03), F(1,29) = 4.9 (P = 0.04), and F(1,26) = 4.5 (P = 0.04), respectively. However, at the maximal doses such as 3 and 5 μM, there were no significant differences between adolescent and adult rats with F(1,26) = 3.5 (P = 0.07) and F(1,24) = 0.8 (P = 0.37), respectively. The EC50 for adolescent rats was 0.16 ± 0.02 μM (n = 13), whereas EC50 for adult rats was 0.24 ± 0.04 μM (n = 11). The EC50 values were significantly different between adolescent and adult rats (P = 0.04).

Recently, using double patch techniques on CA1 pyramidal neurons and CB1 receptor-containing interneurons [i.e., cholecystokinin (CCK)-containing basket cells], Neu et al. (2007) reported that GABA release from CA1 CCK containing basket
cells is under homosynaptic tonic inhibition by endocannabinoids. Similarly we observed in some case that AM251, a CB1 specific agonist, reversed the effect of WIN55,212-2 above the baseline, suggesting the tonic effect of endogenous cannabinoids on the evoked IPSCs. We observed the tonic endogenous effects, in 3 of 13 neurons (23%) from adolescent rats (107–110% above baseline) whereas 5 of 12 neurons (42%) from adult rats showed such effects with greater magnitude (107–146% above baseline). However, when we averaged all data, there was no statistically significant difference between adolescent CA1 neurons (94.3 ± 3.3%, n = 13) and adult CA1 neurons (103.6 ± 6.2%, n = 12; Fig. 3E).

We further examined the effect of AM251 on sIPSCs. AM251 increased the frequency of sIPSCs along with the increase in the amplitude of sIPSCs (Fig. 4, A and B), suggesting the presence of tonic cannabinoid effects. We observed tonic cannabinoid effects in 3 of 12 (25%) CA1 neurons from adolescent rats (K-S tests, P < 0.01), whereas 5 of 12 (42%) CA1 neurons from the adult rats showed the tonic cannabinoid effects (K-S tests, P < 0.01). When we compared the averaged frequency of sIPSCs, AM251 significantly increased the frequency of sIPSCs in adult CA1 neurons (P < 0.01) but not adolescent CA1 neurons (P = 0.31; Fig. 4C). In addition, the percent change of the mean sIPSC frequency in the presence of AM251 was significantly different between adolescent and adult CA1 neurons (P = 0.02). However, AM251 did not significantly change the mean amplitude of sIPSCs in either adolescent (P = 0.48) or adult (P = 0.65) CA1 neurons. This result suggests that the inhibitory neurotransmission in adult CA1 neurons is under greater tonic inhibition by endocannabinoids compared with adolescent CA1 neurons.

We examined the effect of exogenous CB1 receptor agonist, WIN55,212-2, on excitatory neurotransmission in the CA1 pyramidal cells from adolescent and adult rats. WIN55,212-2 (1 μM) significantly decreased the amplitude of evoked EPSCs in CA1 pyramidal cells from adolescent and adult rats, and this effect was reversed by AM 251, a specific CB1 receptor antagonist, confirming that the agonist effect was mediated through CB1 receptors (Fig. 5). However, the magnitude of the effect of WIN55,212-2 on evoked EPSCs did not differ significantly between neurons from adolescent and adult rats (P = 0.73). WIN55,212-2 (1 μM) decreased evoked EPSC by 46.9 ± 3.6% (n = 7) in neurons from the adolescent rats, whereas WIN55,212-2 decreased evoked EPSC by 45.1 ± 3.9% (n = 7) in neurons from the adult rats. WIN55,212-2 also increased the paired-pulse ratio, and this effect was reversed by
AM 251, confirming the presynaptic mechanism of WIN55,212-2. WIN55,212-2 (1 μM) increased the paired-pulse ratio from 1.4 to 1.8 (24.7 ± 1.7%, \( P < 0.01 \), \( n = 7 \)) in neurons from the adolescent rat, whereas WIN55,212-2 (1 μM) increased paired-pulse ratio from 1.4 to 1.75 (25.9 ± 3.2%, \( P < 0.01 \), \( n = 7 \)) in neurons from the adult rat. There was no significant difference in the magnitude of the effect of WIN55,212-2 on paired-pulse ratio between adolescent and adult rats (\( P = 0.74 \)).

**DISCUSSION**

The main finding of this study is that CB1 receptor-mediated synaptic function in the hippocampus is developmentally regulated with significant differences between effects in hippocampi from adolescent rats compared with adult rats. We found that endocannabinoid-mediated I-LTD were of greater magnitude in hippocampal slices from adolescent animals as was the suppression of evoked IPSCs by the CB1 agonist, WIN55,212-2. In contrast, although WIN55,212-2 affected evoked EPSCs, the magnitude of this effect was not different in neurons from adolescent and adult animals. This finding is consistent with those of Kawamura et al. (2006), who reported that the effect of CB1 receptor activation on the excitatory synaptic activity in the hippocampus was not different in mice 27-39 days old compared with those >84 days old. However, it is difficult to compare our results with other previously published data because most studies have been done only during early development and have not included studies of neurons from adult animals (Chevaleyre and Castillo 2003; Hoffman et al. 2003; Mato et al. 2004).

Early studies suggested that excitatory neurotransmission was either insensitive to cannabinoids or sensitive to cannabinoids through mechanisms other than CB1 receptor activation (Hajos and Freund 2002). However, more recent studies have demonstrated the existence of CB1 receptors on excitatory terminals as well as the CB1 selective modulation of excitatory neurotransmission. (Domenici et al. 2006; Katona et al. 2006; Kawamura et al. 2006; Takahashi and Castillo 2006). This is consistent with our finding that a cannabinoid agonist, WIN55,212-2, suppressed the glutamatergic neurotransmission in the hippocampus and that this response was reversed by the selective CB1 antagonist, AM251. The magnitude of suppression of excitatory neurotransmission by WIN55,212-2 was not different in neurons from adolescent and adult animals, a finding that is consistent with those of Kawamura et al. (2006).

The mechanism underlying this age-related decrease of the cannabinoid sensitivity on inhibitory neurotransmission is not clear yet. The lack of developmental regulation of the cannabinoid sensitivity of excitatory neurotransmission excludes the possibility that the developmental differences we observed were due to developmental differences in drug accessibility to the neurons. Developmental regulation of inhibitory neurotransmission could be due to differences in CB1 receptor numbers and/or the functional coupling between cannabinoid receptors and G proteins on inhibitory neuron terminals in adolescence compared with adulthood. Regarding receptor ontogeny, Rodriguez de Fonseca et al. (1993) reported that

**FIG. 5.** WIN55,212-2 decreases the evoked fast EPSCs (non-NMDA receptor-mediated) in CA1 hippocampal area to a comparable magnitude in slices from adolescent rats than from adult rats. A: average time-dependent responses from adolescent (○, \( n = 7 \)), and adult (●, \( n = 7 \)) rats. B: average magnitudes of WIN55,212-2 effects on evoked EPSCs and from adolescent (□) and adult (●) rats. The effect of WIN55,212-2 on evoked EPSCs in slices from adolescent rats (46.9 ± 3.6%, \( P < 0.01 \)) was comparable to that from adult rats (45.1 ± 3.9%, \( P < 0.01 \); \( P = 0.73 \) between groups). These effects were reversed by AM251. C: representative evoked EPSCs before (control), 10–15 min after WIN55,212-2 application (WIN), 20–25 min after WIN55,212-2, and AM251 (AM251) from adolescent (a) and adult (b) rats. Traces of CON and WIN were scaled and superimposed in the last panel. EPSCs were evoked in pairs with 50-ms intervals. Scale bars are 1 nA and 25 ms. D: averaged results on paired-pulse ratio in adolescent (○) and adult (●) hippocampal slices. Notice paired-pulse ratio is increased by WIN and reversed by AM251. The enhancement of paired-pulse ratio was comparable in the adolescent and adult hippocampus (\( P = 0.74 \)).
CB1 receptors are present early in ontogeny (2 and 5 days after birth) in rats and that the receptor level was maximal on days 30 or 40 (during the adolescent period) and then decreased to adult values. Other receptor studies have reported values at weaning (day 21) and in adulthood (Belue et al. 1995; Mclaughlin et al. 1994) but did not address the possibility of a peak during adolescence followed by adult pruning, analogous to dopamine receptor numbers in the forebrain (Anderson et al. 2000; Teicher et al. 1995). Interestingly, Mata and Pazos (2004) reported a significant decrease in CB1 receptor density and CB1 agonist-stimulated G-protein activity with age, revealing a mean reduction of 10% per decade in adult human frontal cortex, whereas Wang et al. (2003) observed regionally selective reduction in cannabinoid-stimulated GTP(γS) labeling in aged mice, compared with young adult mice, without any measurable difference in the receptor level between the age groups.

Alternatively, the different sensitivity to exogenous cannabinoids in the adolescent and adult CA1 neurons could be due to different tonic inhibition by endogenous cannabinoids between two age groups. Tonic inhibition by endocannabinoids on inhibitory neurotransmission has been demonstrated in the several brain regions such as the hypothalamus, amygdala, and hippocampus (Hentges et al. 2005; Losonczy et al. 2004; Neu et al. 2007; Zhu and Løvinger 2005). In this study, we observed that inhibitory inputs onto CA1 pyramidal neurons are tonically suppressed by endocannabinoids and that these tonic endocannabinoid effects were greater in the adult CA1 neurons compared with adolescent CA1 neurons. The greater tonic inhibition by endocannabinoids in the adult CA1 neurons could lead to less sensitivity to exogenous cannabinoid-mediated inhibition on inhibitory neurotransmission. It remains to be explored whether other brain regions such as amygdala and hypothalamus also show the increase in the tonic cannabinoid effects in the adult animals.

Tonic inhibition of inhibitory neurotransmission by endogenous cannabinoids was shown to be mediated through the tonic release of endogenous cannabinoids rather than constitutive activation of CB1 receptors (Neu et al. 2007). However, not all CB1 receptor-containing synapses are sensitive to this tonic inhibition, raising the question on its specificity. In addition, the magnitude of tonic inhibition by endogenous cannabinoids vary depending on synapses, and at certain synapses, it has been shown to silence the connection of CB1 containing interneurons to the pyramidal neurons (Losonczy et al. 2004; Neu et al. 2007). Therefore any physiological or pathological changes to decrease the tonic endocannabinoid function could change neuronal network by recruiting this previously muted connection between CB1 containing interneurons and pyramidal neurons. On the other hand, any changes to increase the tonic endocannabinoid function could reshape the neuronal network by removing more CB1-containing interneurons from network. Although the functional significance of endocannabinoid-mediated tonic inhibition remains to be determined, it could also dampen endocannabinoid-mediated phasic inhibition, such as DSI or I-LTD, as well as the effects by exogenously applied cannabinoids such as marijuana in a self-limiting way.

Only a subset of GABAergic interneurons, i.e., CCK-containing basket cells, expresses CB1 receptors, and these provide perisomatic inputs to pyramidal neurons (Katona et al. 1999; Marsicano and Lutz 1999; Tsou et al. 1999). Pyramidal neurons also have perisomatic inputs from parvalbumin-containing basket cells, which are devoid of CB1 receptors. It has been suggested that parvalbumin-containing basket cells operate as part of a nonplastic network, whereas CCK-containing basket cells are highly modifiable and involved in the fine-tuning of network cooperativity (for review, see Freund 2003). Thus different sensitivity to the cannabinoid-mediated inhibition in CA1 neurons between adolescent and adult rats could lead to significant functional differences such as those that underlie learning and memory.

In conclusion, we observed greater cannabinoid effects on the inhibitory synaptic plasticity and functionality in hippocampal slices from adolescent rats compared with adult rats. There was no developmental regulation of the suppressive effect of the CB1 agonist on excitatory synaptic activity. Greater CB1 sensitivity of hippocampal inhibitory neurotransmission in adolescent rats may represent a neurobiological mechanism underlying the greater sensitivity of adolescent animals to the cognitive impairment associated with acute THC exposure (Cha et al. 2006). These findings provide a plausible mechanism to explain the greater vulnerability of adolescents to the memory impairment caused by smoking marijuana.

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