Cannabinoids Modulate Synaptic Strength and Plasticity at Glutamatergic Synapses of Rat Prefrontal Cortex Pyramidal Neurons

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Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. J Neurophysiol 83: 3287–3293, 2000. Cannabinoids receptors have been reported to modulate synaptic transmission in many structures of the CNS, but yet little is known about their role in the prefrontal cortex where type I cannabinoid receptor (CB-1) are expressed. In this study, we tested first the acute effects of selective agonists and antagonist of CB-1 on glutamatergic excitatory postsynaptic currents (EPSCs) in slices of rat prefrontal cortex (PFC). EPSCs were evoked in patch-clamped layer V pyramidal cells by stimulation of layer V afferents. Monosynaptic EPSCs were strongly depressed by bath application (1 μM) of the cannabinoid receptors agonists WIN55212-2 (−50.4 ± 8.8%) and CP55940 (−42.4 ± 10.9%). The CB-1 antagonist SR141716A reversed these effects. Unexpectedly, SR141716A alone produced a significant increase of glutamatergic CB-1 antagonist SR141716A reversed these effects. Unexpectedly, SR141716A alone produced a significant increase of glutamatergic synaptic transmission (+46.9 ± 11.2%), which could be partly reversed by WIN55212-2. In the presence of strontium in the bath, the frequency but not the amplitude of asynchronous synaptic events evoked in layer V pyramidal cells by stimulating layer V afferents, was markedly decreased (−54.2 ± 8%), indicating a presynaptic site of action of cannabinoids at these synapses. Tetanic stimulation (100 pulses at 100 Hz, 4 trains) induced in control condition, no changes (n = 7/18), long-term depression (LTD; n = 6/18), or long-term potentiation (LTP; n = 5/18) of monosynaptic EPSCs evoked by stimulation of layer V afferents. When tetanus was applied in the presence of WIN 55,212-2 or SR141716-A (1 μM) in the bath, the proportion of “nonplastic” cells were not significantly changed (n = 7/15 in both cases). For the plastic ones (n = 8 in both cases), WIN 55,212-2 strongly favored LTD (n = 7/8) at the apparent expense of LTP (n = 1/8), whereas the opposite effect was observed with SR141716-A (7/8 LTP; 1/8 LTD). These results demonstrate that cannabinoids influence glutamatergic synaptic transmission and plasticity in the PFC of rodent.

INTRODUCTION

Marijuana, a compound well known for its psychoactive properties, has also significant analgesic, anti-emetic and appetite stimulant effects, which are of interest for cancer and AIDS patients (Weinroth et al. 1995). The major psychoactive component of marijuana, △9-tetrahydrocannabinol (∈THC), also impairs some cognitive processes including memory (Abood and Martin 1992). Effects of cannabinoids are thought to be mediated through the activation of receptors negatively coupled to adenylate cyclase, the so-called type 1 (CB-1) and type 2 (CB-2) cannabinoid receptors. The latter is mainly localized in the peripheral immune system and is largely absent from brain (Galiegue et al. 1995; Munro et al. 1993; Pertwee 1997), whereas the former is widely expressed in the CNS (see references in Bidaut-Russell et al. 1990; Matsuda et al. 1993) and in several peripheral tissues (Galiegue et al. 1995).

Besides inhibiting N- and Q-type voltage-gated calcium (Ca) channels and enhancing inwardly rectifying A-type potassium (K) channels in expression systems and in cultured hippocampal neurons (see references in Childers and Deadwyler 1996; Deadwyler et al. 1993; Mackie et al. 1995), CB1 receptors acutely depress glutamatergic synaptic transmission through a presynaptic mechanism in the hippocampus and in the cerebellum (Levenes et al. 1998; Shen et al. 1996). Activation of CB1 receptors also impairs induction of long-term potentiation (LTP) in the hippocampus (Nowicky et al. 1987; Stella et al. 1997; Terranova et al. 1995) and long-term depression (LTD) in the cerebellum (Levenes et al. 1998).

Despite the fact that CB-1 receptors are less expressed in the prefrontal cortex (PFC) of rodents than in cerebellum, hippocampus, or basal ganglia (Herkenham et al. 1991; Tsou et al. 1998), the density of cannabinoid receptors in this structure is relatively high compared with other G-protein–coupled receptors (Herkenham et al. 1990). Accordingly, △9THC in the PFC of rodents has been reported to induce suppression of spatial working memory (Jentsch et al. 1997, 1998). Since PFC is also involved in cognitive and memory functions (Aggleton et al. 1995; Eichenbaum et al. 1983; Ragozzino et al. 1998; Seamans et al. 1995), we decided to study the effects of the selective cannabinoid receptor agonists WIN 55212-2 and CP 55940 (Devane et al. 1988), and of the selective antagonist SR141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4- dichlorophenyl)-4-methyl-1H-pyrazol-3-carboxamide hydrochloride] (Rinaldi-Carmona et al. 1994) on synaptic transmission and synaptic plasticity in this structure. Indeed, long-term changes in synaptic efficacy are generally considered as the cellular basis of learning and memory. The study was focused on layer V pyramidal cells since they can express both LTP and LTD of glutamatergic synaptic transmission (Hirsch and Crepe 1990).

METHODS

Brain slice preparation

Coronal brain slices from 17- to 22-day-old male Sprague-Dawley rats containing the prelimbic area of PFC (300 μm; 2.2–3.7 mm from bregma) were sectioned with a vibratome in chilled (≈0°C) oxygen-
ated (95% O2; 5% CO2) artificial cerebrospinal fluid (ACSF) of the following composition (mM): 124 NaCl, 3 KCl, 24 NaHCO3, 1.15 KH2PO4, 1.15 MgSO4, 2 CaCl2, and 11 glucose, final pH 7.35, 325 mosmol/l at 28°C. The slices were allowed to recover for at least 2 h at room temperature (≈20°C) in a chamber filled with continuously oxygenated ACSF. A slice was then transferred to a submerged-type recording chamber and continuously perfused with oxygenated ACSF (28°C) containing 1 μM of bicuculline methochloride (GABA_A antagonist; Sigma) at the rate of 2 ml/min.

**Recording and stimulation**

Layer V pyramidal neurons in prelimbic area of PFC were identified initially by videomicroscopy with Nomarski optics under infrared illumination through the ×40 water immersion objective (Stuart et al., 1993) as well as by biocytin staining performed after experiments. Excitatory postsynaptic currents (EPSCs) were recorded in whole cell voltage-clamp mode with a patch-clamp amplifier (Axopatch 200A, Axon Instruments) and filtered at 2 kHz. Electrodes (2–4 MΩ) were filled with a solution containing (mM) 8 NaCl, 100 potassium gluconate, 40 KCl, 10 HEPES, 0.5 EGTA, and 26 biocytin (Sigma); pH 7.24, 295 mosmol/l. The mode of spike discharge was routinely examined in current-clamp mode by application of a 0.5-nA depolarizing current step (200 ms). Neurons were classified as regular spiking, burst spiking, and irregular spiking [70, 17, and 13% of the recorded cells, respectively, following in principle the classification made by Law-Tho et al. (1995)], and no correlation has been found between a discharge mode, the sense of plasticity induction, or the CB-1 agonists/antagonist effects. Cells were then held at −70 mV after a partial compensation (80%) of access resistance. A monopolar stimulating electrode was placed in layer V, 10–30 μm laterally to the apical dendrite of the recorded cell. The EPSCs were elicited at 0.1 Hz on 10-mV hyperpolarizing voltage steps that allowed monitoring of the passive electrical properties of the recorded cell throughout the experiment. For plasticity experiments, after a baseline period of at least 8 min, tetanic stimuli consisting of 4 trains of 100-Hz burst (100 pulses) were delivered at 0.1 Hz under current-clamp mode. The voltage-clamp mode and the baseline stimulation were restored 10 s after the end of the last tetanic stimulation.

**Pharmacological treatment**

Drugs used were as follows: SR141716A (a generous gift from Sanofi Recherche, Montpellier, France), WIN 55212-2 (Tocris Cookson), and CP 55940 (Tocris Cookson). Stock solutions of SR141716A, WIN 55212-2, or CP 55940 (10 mM in dimethylsulfoxide; DMSO) were dissolved in ACSF at right concentrations (see RESULTS); a sonicator facilitated dissolving of these DMSO solutions. In pilot experiments (n = 10), we ascertained that the final concentration of DMSO in the bath (0.1–0.2%, depending on experiments) had no detectable effects on bioelectrical or synaptic activities of pyramidal neurons.

**Data collection and analysis**

Digitized data were acquired and analyzed on-line and off-line with AcquisI software (Gérard Sadoc, Center National de la Recherche Scientifique, Gif-sur-Yvette, France). The initial slope of the EPSCs (a 1-ms period from its onset; pA/ms), which contains only the monosynaptic component of the responses (Hirsch and Crepel 1990), was used to determine changes in synaptic efficacy of monosynaptic afferents impinging on layer V pyramidal cells. For this purpose, all responses were normalized to the mean initial slope recorded during a 10-min control period. Cells were considered as exhibiting increase/decrease for acute effects of compounds or LTP/LTD for plasticity experiments, when changes in synaptic efficacy in any direction was found significant with ANOVA test (P < 0.05). Values are expressed as means ± SE.

For analysis of the evoked quantal events in the presence of strontium (Sr2+), synaptic currents were further filtered at 2 kHz and analyzed off-line with Detectivent, Labview-based software developed by N. Ankr (Unité INSERM U261 and Institut Pasteur, Paris). synaptic events were detected on the basis of the slope of their rising phase, and of windows for allowed minimum/maximum times to peak and decay time constants (Ankr et al. 1994). These parameters were adjusted so that most visible synaptic events were detected. For each cell, analysis was performed on a few hundred of such selected evoked synaptic events. Although the method of detection did not take into account absolute detection thresholds for evoked quantal EPSC amplitudes, it is clear from raw and cumulative amplitude distributions that events smaller than 10 pA were probably generally not detected, most of them therefore being likely to be buried in the background noise.

**Biocytin staining**

Recordings with biocytin-containing electrodes were made from one cell per slice to verify that it was a pyramidal cell in layer V that was recorded. Slices were then fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C and rinsed three times (10 min each) in PBS. The biocytin was revealed by preincubating the slices with Vectastain ABC standard peroxidase (Valbiotech) and 0.1% Triton X-100 (Sigma) for permeabilization in PBS for 4 h. Slices were then rinsed three times (10 min each) before being incubated with peroxidase kit substrate (Valbiotech) for 10 min. The reaction was stopped by rinsing again three times (10 min each) with PBS, and the slices were mounted in PBS 50%/glycerol 50%. Pyramidal neurons were visualized with a ZEISS axiovert microscope, and images were taken using a camera (Hamamatsu) mounted on the microscope.

**RESULTS**

The results of the present study were obtained from 119 pyramidal cells. An example of one of those cells is illustrated in Fig. 1A. Input resistance, holding current (i.e., <200 pA at −70 mV), and synaptic responses were checked during baseline acquisition.

**EPSCs evoked in layer V pyramidal cells by stimulation of layer V**

At −70 mV, all pyramidal cells responded to layer V stimulation with a short latency (range: 2–4 ms) inwardly directed postsynaptic current (PSC). Superfusion with low doses of bicuculline methochloride (1 μM) significantly decreased (P < 0.05; n = 3; Wilcoxon test) the PSC amplitude measured at −70 mV (Fig. 1B). In the presence of bicuculline, holding the cell at progressively more depolarized potentials revealed an apparent reversal potential for the EPSC of −3.2 ± 6 mV (n = 5; Fig. 1, D and E). On the other hand, in the absence of bicuculline, bath application of 5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 30 μM 2-amino-5-phosphonovaleric acid (APV) revealed an inhibitory postsynaptic current (IPSC) component with an apparent reversal potential of −35.5 ± 4 mV because of the presence of 40 mM KCl in the micropipettes (data not shown). These results confirm the participation of EPSCs and IPSCs in the recorded PSCs. On the whole, PSCs evoked by layer V stimulation in layer V pyramidal cells were therefore very similar to those evoked by
stimulation of more superficial layers in previous studies (Hirsch and Crepel 1990; Otani et al. 1998). In subsequent experiments, cells were bathed with 1 mM bicuculline methochloride to isolate the EPSC component. The initial rising slope of the EPSC was measured to follow the monosynaptic component of the EPSC (Fig. 1C) and avoid any contamination by the polysynaptic component.

Acute effects of cannabinoid agonists and antagonists on monosynaptic EPSCs

In three of the four cells tested, bath application of the CB-1 agonist WIN 55212-2 (1 μM) for 10 min induced a significant decrease of the initial slope of monosynaptic EPSCs to −50.4 ± 8.8%, which lasted thereafter for the whole duration of experiments, i.e., usually up to 30 min (Fig. 2A). In the remaining cell, no effect was observed. In four of six other cells, a similar effect was obtained with bath application of the CB-1 agonist CP 55940 (1 μM), which also induced an apparently irreversible and significant decrease of the initial slope of the EPSCs (42.4 ± 10.9%). Again, in the two remainders, no effect was observed. The depressant effect of cannabinoids on EPSCs was never accompanied by any detectable changes in passive bioelectrical properties of the cells or in their holding current.

We next determined whether bath application of the CB-1 antagonist 1 μM SR141716A alone has any effects on the amplitude and kinetics of the EPSC. In marked contrast with previous studies (Collins et al. 1995; Levenes et al. 1998; Shen et al. 1996; Terranova et al. 1995), bath application of SR141716A (10 min) induced, in four of the nine tested cells, a significant increase of the initial slope of the EPSC, which amounted to +46.9 ± 11.2% and also lasted thereafter for the whole duration of experiments (Fig. 2B). In the five remaining cells, however, no significant effect of SR141716A on the EPSCs was observed. In no cell, detectable changes in passive

![Fig. 1.](http://jn.physiology.org/)

**Fig. 1.** A: example of layer V pyramidal cell visualized by biocytin staining. B: synaptic current recorded at −70 mV in artificial cerebrospinal fluid (ACSF; 1) and in ACSF with 1 mM of bicuculline (2), generated by stimulating of layer V. C: same trace as in B (2) showing the initial rising slope of the monosynaptic component of the excitatory postsynaptic current (EPSC). D: in another cell, synaptic currents generated at different holding membrane potentials with bicuculline (1 μM) in the bath to reveal the EPSC component. E: current-voltage (I-V) relation of amplitude of EPSC measured at 4.1 ms delay (↓) after stimulus artifact. The I-V relation is almost linear and shows a reversal potential of 2 mV for the EPSC.

![Fig. 2.](http://jn.physiology.org/)

**Fig. 2.** A, top traces: superimposed averaged EPSCs recorded before (1) and after (2) the depressant effect of WIN55212-2 (1 μM) in a representative cell. A, bottom: plot of normalized EPSC slope against time (mean ± SE), before, during, and after bath application of 1 μM WIN55212-2. B, top traces: superimposed averaged EPSCs recorded before (1) and after (2) the potentiating effect of SR141716A (1 μM). B, bottom: plot of normalized EPSC slope against time (mean ± SE) before, during, and after bath application of 1 μM SR141716A (n = 4). C, top traces: superimposed averaged EPSCs recorded before (1) and after (2) the depressant effect of WIN55212-2 and reversal (3) of the effect by SR141716A in a representative cell. C, bottom: plot of normalized EPSC slope against time in the same conditions (n = 6). D, top traces: partial reversal by WIN55212-2 of the potentiating effect of SR141716A shown as superimposed averaged EPSCs recorded in one cell. D, bottom: same effect shown as the plot of normalized EPSC slope against time (n = 4).
bioelectrical properties or their holding current were noted during application of SR141716A.

In separate experiments (n = 6), bath application of 1 μM SR141716A fully or partly reversed the depressant effect of 1 μM WIN 55212-2 on monosynaptic EPSCs, when WIN 55212-2 was washed out at the same time as SR141716A was applied (Fig. 2C). In contrast, in four other cells, when perfusion of WIN 55212-2 was maintained during SR141716A application, no reversal of the depressant effect of WIN 55212-2 on EPSCs was observed (data not shown).

Since SR141716A by itself potentiated the EPSCs, we ascertained whether such an effect could be also antagonized by WIN 55212-2. Indeed, in four of six other cells where the EPSCs were potentiated by bath application of 1 μM SR141716A, a subsequent application of 1 μM WIN 55212-2 during wash out of the SR141716A partly or fully reversed the potentiation of the EPSCs (Fig. 2D). Here again, in four other cells where perfusion of SR141716A was maintained during WIN 55212-2 application, no such reversal of the potentiating effect of SR141716A on EPSCs was observed (data not shown). Altogether, these results demonstrate that interactions between WIN 55212-2 and SR141716A occur specifically at the level of CB-1 receptors. The reversals of the effects do not simply represent algebraic summation of their opposite effects on EPSCs.

Site of action of agonist cannabinoids

To determine whether the depressant effect of cannabinoids on EPSCs has a pre- and/or postsynaptic origin, we have substituted the Ca2+ in the bathing medium by Sr2+. This treatment is known to induce a reduction of the stimulation-evoked synchronous release of transmitter, and selectively enhance asynchronous release of quanta, thus allowing detailed analysis of evoked quantal events (Miledi 1966; Oliet et al. 1996). In the six cells tested, this substitution led to a marked decrease in the amplitude of the EPSCs (Fig. 3A) and to the appearance of numerous asynchronous quantal events, which lasted for a few hundred milliseconds after the stimulus. Figure 3B illustrates such asynchronous events recorded from one of the six cells tested in control condition and in the presence of WIN55212-2 (1 μM) in the bath. As can be seen in this typical example, WIN55212-2 markedly reduced both the amplitude of the residual EPSC, and the frequency of occurrence of subsequent asynchronous evoked quantal events. Similar results were observed in four of these six cells, WIN55212-2 having no detectable effects on the two others. In these four cells kinetics of evoked quantal events were not affected by WIN55212-2 (Fig. 3C). We next compared for these cells, the raw and the cumulative amplitude distribution of the evoked asynchronous EPSCs that occurred during a 400-ms time window after stimulation in the presence or in the absence of WIN55212-2 in the bathing medium. Neither the raw amplitude, nor the cumulative amplitude distribution (n = 4; Kolmogorov-Smirnov test, P < 0.05) are affected by the application of WIN55212-2 (Fig. 3, D and E). In contrast, such WIN55,212-2 in strontium medium significantly decreased in four of the six cells (n = 4; Student’s paired t-test; P < 0.05) the mean detection rate of the events from 20.8 ± 4.1 (SE) Hz in control conditions to 9.55 ± 0.8 Hz (i.e., −54.2 ± 8%; Fig. 3F). These results strongly suggest that the site of action of

WIN55212-2 on layer V afferents mediated EPSCs is entirely presynaptic.

Changes in synaptic efficacy induced by tetanization of layer V

In control medium (i.e., 1 μM bicuculline methochloride in the bathing medium), tetanic stimulation applied after a control period of at least 10 min (see METHODS) induced no long-lasting changes in the initial slope of the monosynaptic EPSCs in 7 of 18 tested cells. In the 11 others, either a clear LTP (mean

FIG. 3. A: averaged layer V afferents mediated EPSCs (n = 20) recorded in a pyramidal cell in standard ACSF with 1 μM of bicuculline (control) and after Sr2+ was substituted for Ca2+ (Sr2+). B: examples of evoked asynchronous events recorded in this pyramidal cell in control solution (Sr2+ control) and in the same medium containing 1 μM of WIN55212-2 (Sr2+ WIN). Note the reduction of the amplitude of the residual stimulation-evoked synchronous EPSCs in Sr2+ control and in Sr2+ WIN. C: averaged (n = 20) Sr2+-induced asynchronous EPSCs in control conditions and in the presence of WIN55212-2, in the same cell. D: superimposed histograms of the amplitude distributions of Sr2+-induced asynchronous EPSCs for the cell illustrated in A–C, under control conditions and in the presence of WIN55212-2 in the bath. E: cumulative mean amplitude distributions of asynchronous events in these 2 conditions (n = 4). F: histogram of mean detection rate (±SE) of asynchronous events in Sr2+ control and in Sr2+ WIN. Detection rates are normalized relative to the mean detection rate in the control solution.

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increase of the initial slope is $-34.3 \pm 8.3\%$; $n = 5$; Fig. 3A) or a clear LTD (mean decrease of the initial slope is $+33.0 \pm 7\%$; $n = 6$; Fig. 4B) of the monosynaptic EPSCs was induced. These LTP and LTD lasted as long as the cells were recorded, usually up to 40 min. Thus in control medium, about one-third of the cells were not plastic, and, for the other two-thirds, LTP usually up to 40 min. Thus in control medium, about one-third of the cells were not plastic, and, for the other two-thirds, LTP usually up to 40 min. Thus in control medium, about one-third

When WIN 55212-2 (1 μM) was added to the perfusion medium 10 min prior to the recording session (i.e., 20 min before tetanus), synaptic plasticity was markedly shifted in favor of LTD. Indeed, in 15 cells tested in this condition, only 1 cell exhibited LTP of the EPSC, with an increase of the initial slope of $+27.4\%$, whereas 7 others exhibited LTD of the monosynaptic EPSC, with mean decrease of the initial slope $36.5 \pm 3.3\%$ (Fig. 4C). The other seven cells were not plastic.

Conversely, when SR141716A (1 μM) was added to the bath 10 min before recording the cells, synaptic plasticity induced by tetanic stimulation was now markedly shifted in favor of LTP. Thus in 15 cells tested in such a condition, again seven cells exhibited no change in initial EPSC slope. For the eight others, only one exhibited LTD of the EPSC, with a decrease of the initial slope of $43.2\%$, whereas the remaining seven exhibited LTP of the EPSC, with mean increase of the initial slope $34.7 \pm 9\%$ (Fig. 4D).

Interestingly, in both experiments with SR141716A and with WIN 55212-2, the proportion of nonplastic cells was nearly the same and not statistically different from the proportion of nonplastic cells in control medium (Fig. 5). On the other hand the proportion of LTD and LTP under SR141716A compared with those obtained under WIN55212-2 were statistically different ($\chi^2$ test, $P < 0.05$). The results suggest that nonplastic cells are not rendered plastic by WIN 55212-2 or by SR141716A, and that those two compounds induce a shift in synaptic plasticity in the same population of plastic neurons or synapses.

Finally, WIN 55212-2 and SR141716A mutually inhibited their effects on synaptic plasticity. Thus in 14 tested cells, when both compounds were added to the perfusing medium 15 min prior to the tetanus, LTP and LTD were induced in five and four cells, respectively, i.e., in proportions, not different to those obtained in control medium (Fig. 5).

FIG. 5. Proportions of nonplastic cells, cells exhibiting LTD, or cells exhibiting LTP in 4 different conditions; control, WIN, SR, or both WIN + SR in the bath. For the plastic cells ($n = 8$ in both cases WIN or SR), WIN 55212-2 strongly favored LTD ($n = 7/8$) at the apparent expense of LTP ($n = 1/8$), whereas the opposite effect was observed with SR141716-A ($7/8$ LTD; 1/8 LTP). The proportion for LTD and LTP under WIN + SR conditions was not significantly different from that in control condition. *$\chi^2$ test indicating that the proportions of LTD and LTP under WIN55212-2 are statistically different from those found under SR141716A ($P < 0.05$).

Discussion

The present study demonstrates that, in rat PFC, cannabinoid receptor agonists inhibit glutamatergic synaptic transmission between layer V afferents and layer V pyramidal cells via a presynaptic action, and favor LTD at the expense of LTP at the same synapses. More importantly, the present study also establishes for the first time that the specific cannabinoid receptor antagonist SR141716A acutely increases glutamatergic transmission and favors LTP at the expense of LTD.

The acute effects of cannabinoid agonists on excitatory synaptic responses are consistent with recently published studies in hippocampal glutamatergic transmission in cultures (Shen et al. 1996) and in the cerebellum (Levenes et al. 1998), although in our case, some cells did not respond to these compounds. This could be explained by a poor diffusion of cannabinoids through slices, due to their lipophilic nature, which probably decreases their actual concentrations at the

FIG. 4. A and B, top traces: superimposed averaged EPSCs recorded before (1) and after (2) tetanic stimulation in 2 different cells, one (A) expressing a long-term potentiation (LTP) and the other (B) a long-term depression (LTD), respectively. A, bottom: plot of normalized EPSCs slope against time before and after tetanic stimulation (1) for cells expressing LTP or (B, bottom) LTD. C, top traces show superimposed averaged EPSCs recorded before (1) and after (2) tetanic stimulation, and the bottom represents the plot of normalized EPSC slope against time of the 6 cells exhibiting LTD in the presence of WIN55212-2. D: same conditions as in C except that SR141716A was applied in the bath. Conversely, with SR141716A in the bath, the same tetanic stimulation induced preferentially LTP ($n = 5$).

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level of CB-1 receptors. Higher concentrations could not be tested because the drugs were dissolved in DMSO, which induced by itself a decrease of the synaptic responses at concentration higher than 0.2%. The fact that WIN 55212-2 and CP 55940, two specific CB-1 receptor agonists (Devane et al. 1988) have the same acute depressant effect on EPSCs, and that WIN 55212-2 and the specific cannabinoid receptor antagonist SR141716A mutually inhibit their respective effects on EPSCs and synaptic plasticity (see RESULTS) confirm that all these effects are genuinely mediated through the activation of CB-1 receptors. The decrease by WIN55212-2 of the frequency of evoked quantal synaptic events, without affecting their amplitude, strongly suggests that cannabinoids exert their effects on monosynaptic EPSCs through a presynaptic mechanism, which is also in agreement with results in other structures (Levenes et al. 1998; Shen et al. 1996).

The impairment of LTP in layer V pyramidal cells by WIN 55212-2 is in keeping with the inhibition by cannabinoid agonists of LTP in the hippocampus (Nowicky et al. 1987; Stella et al. 1997; Terranova et al. 1995) and of LTD in the cerebellum (Levenes et al. 1998). In the present study, this impairment does not result from a general impairment of synaptic plasticity but rather represents a shift of long-term changes in synaptic efficacy toward LTD (see RESULTS). In this respect, the acute and long-term effects of cannabinoid agonists on glutamatergic synaptic transmission in layer V pyramidal cells of the rat PFC are reminiscent of those observed in the same cells by dopamine (DA) (Law-Tho et al. 1995; Otani et al. 1998). Interestingly, CB-1 receptors and D2 dopaminergic receptors are both known to be negatively coupled to adenylyl cyclase in rat brain (Bidaut-Russell et al. 1990; Jaber et al. 1996). Now, are the present results consistent with the theory of synaptic plasticity developed by Bienenstock, Cooper, and Munro and referred to as the BMC theory (Bienenstock et al. 1982)? If one considers that cells exhibiting no long-lasting change in synaptic efficacy are, for some reason, nonplastic in our experimental conditions (see RESULTS), the present results as well as those of our previous studies with DA (Law-Tho et al. 1995; Otani et al. 1998) are consistent with the view that both cannabinoid agonists and DA shift the floating threshold defined by the BMC theory toward more depolarized values, and thus favor LTD at the expense of LTP.

Two alternative hypotheses can explain the potentiating effects of SR141716A on synaptic transmission and the shift of synaptic plasticity in favor of LTP at the expense of LTD. The first one is that this effect results from inverse agonist properties of SR141716A (Bouaboula et al. 1997; Landsman et al. 1997; Pan et al. 1998). The second one is that glutamatergic EPSCs evoked in layer V pyramidal cells are tonically inhibited by endogenous cannabinoids. Clearly, the first hypothesis cannot be ruled out on the basis of the present results. However, we favor the second one for several reasons. Indeed, if inverse agonist properties have been reported in heterologous expression system and in pelvic ganglion neurons (Pan et al. 1998), they have not been reported in native neurons in the cerebellum (Levenes et al. 1998) and in the hippocampus (Shen et al. 1996) where apparently the same CB1 receptors as in the PFC are represented (Rinaldi-Carmona et al. 1996). On the other hand, if effects of SR141716A on synaptic transmission and synaptic plasticity were due to inverse agonist properties of the compound, one would expect to have observed these effects as consistently as those of WIN55212-2, given their similar lipophilic nature and similar affinities for CB1 receptors, which was not the case. On the contrary, the fact that the effects of SR141716A were less consistently observed than those of WIN55212-2 fits well with the presence of endogenous cannabinoids, if one simply assumes that their concentration in slices may vary depending on the physiological state of the animal. Finally, Piomelli’s group found an activity-dependent release of the endocannabinoid anandamide that binds to cannabinoid receptors in the striatum (Giuffrida et al. 1999), which also argues in favor of an endogenous cannabinoid release. Further studies will be performed to clarify the issue, as for instance looking at the effects of anandamide uptake blockers on synaptic transmission and synaptic plasticity in layer V pyramidal cells.

In conclusion, the present study brings compelling evidence for a major role of cannabinoids in controlling glutamatergic synaptic transmission and plasticity in rat PFC. These findings are of prime importance, given the role of PFC in higher cognitive processes and memory function, as well as its close association with reward system. Finally, the shift in favor of LTP induced by SR141716A might account for the promnesic effects of SR141716A (Terranova et al. 1996), which makes this compound a candidate of potential interest in the treatment of memory disorders and other cognitive deficits.

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REFERENCES


SYNAPTIC EFFECTS OF CANNABINOIDS IN PREFRONTAL CORTEX


GIFFORD AN AND ASHBY CR Jr. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. J Pharmacol Exp Ther 277: 1431–1436, 1996.


