CB1 Cannabinoid Receptor Inhibits Synaptic Release of Glutamate in Rat Dorsolateral Striatum

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Gerdenman, Gregory and David M. Lovinger. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85: 468–471, 2001. CB1 cannabinoid receptor antagonists have been shown to inhibit neurotransmitter release in various brain areas, and we investigated the effects of CB1 activation on glutamatergic synaptic transmission in the dorsolateral striatum. CB1 agonists HU-210 (1 μM) significantly reduced the frequency with no change in amplitude. These findings further support the interpretation that CB1 activation leads to a decrease of glutamate release from afferent terminals in the striatum. These results reveal a novel potential role for cannabinoids in regulating striatal function and basal ganglia output and may suggest CB1-targeted drugs as potential therapeutic agents in the treatment of Parkinson’s disease and other basal ganglia disorders.

INTRODUCTION

The striatum is the major input nucleus of the basal ganglia, which is important both for the normal control of movements and for the pathophysiology of prevalent movement disorders, such as Parkinson’s disease and Huntington’s disease (Calabresi et al. 1996). Cannabinoids such as Δ9-THC can profoundly influence motor behavior in rodents (Sañudo-Peña et al. 1999) and may affect movement and motivational states in humans (Hollister 1986). High expression levels of the CB1 cannabinoid receptor within the striatum and its target nuclei (Herkenham et al. 1991; Tsou et al. 1998), along with other basal ganglia disorders.

METHODS

Methods for striatal slice preparation and whole cell electrophysiology experiments were essentially the same as previously described (Choi and Lovinger 1997a,b). Cortical brain slices (350–400 μm) containing the cortex and striatum were cut from 14- to 22-day-old Sprague-Dawley rats and maintained at room temperature (21–23°C) in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 4.5 KCl, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 1.2 NaH2PO4, and 10 D-glucose, bubbled with 95% O2,5% CO2. For recording, single hemislices were completely submerged and continuously superfused with ACSF at 31–33°C at a flow rate of 1.5–3 ml/min. For Sr2+-substitution experiments, ACSF containing 3 mM SrCl2, 5 mM

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MgCl₂ and 0 CaCl₂ was exchanged for normal ACSF at the beginning of a recording. This Sr²⁺-ACSF also contained picrotoxin and DL-2-amino-5-phosphovaleric acid (APV), 25 µM each, to prevent contamination of recordings by GABA A and N-methyl-d-aspartate (NMDA) receptor-mediated spontaneous synaptic responses. Picrotoxin and APV were not present in normal ACSF, as GABA A and NMDA receptors do not contribute significantly to stimulus-evoked excitatory postsynaptic currents (EPSCs) in the striatum under these conditions (Calabresi et al. 1996; Lovinger et al. 1993; and data not shown).

Whole cell voltage-clamp recordings were made exclusively from cells in the dorsolateral striatum, where CB1 receptor expression is greatest (Herkenham 1991), using an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA). Patch pipettes (2.5–5 MΩ) were filled with internal solution containing (in mM) 120 CsMeSO₃, 5 NaCl, 10 HEPES, 3–5 QX-314 (Br salt), and 1.1 EGTA, 4 ATP (amino-5-phosphovaleric acid (APV), 25 m

Effects of SR alone (25 µM) prevented inhibition of EPSCs by 1 µM HU-210, taken from an experiment similar to that shown in A. Calibration: 10 ms, 100 pA. C: plot of the paired-pulse response ratio (PPR) measured from the cell in A. D: summary histogram from CB1 agonist experiments. Number of cells is shown in parentheses. Effects are shown as percentage of baseline values ± SE. EPSC values are compared statistically to values measured after application of carrier solution (cyt. c) alone (unpaired t-test, *P < 0.05, **P < 0.01). PPR and coefficient of variation (CV) values are compared with mean baseline values within each group (repeated measures, †P ≤ 0.05).
The drugs HU-210 (Tocris-Cookson), WIN 55,212–2 [Research Biochemicals International (RBI)] and SR141716A (RBI/NIMH chemical synthesis program) were diluted from DMSO stock solutions (10 mM) into ACSF, with cytochrome c (Sigma) dissolved as a carrier at 0.5 mg/ml to minimize drug binding to the perfusion apparatus. All control experiments included the carrier and ≥0.01% DMSO.

RESULTS

EPSCs were evoked in voltage-clamped striatal neurons by electrical stimulation of afferent fibers (Fig. 1B). Application of the CB1 agonist HU-210 reduced the amplitude of evoked EPSCs in a dose-dependent manner (Fig. 1). At a concentration of 1 μM, HU-210 reduced peak EPSC amplitude to 45 ± 15% of stable baseline values, which was significantly different from effects of the carrier solution alone (P < 0.01, n = 7). Similar results were found using a structurally dissimilar CB1 agonist, WIN 55,212–2 (Fig. 1D; 55 ± 6% baseline, P < 0.05, n = 4). Agonist treatments did not alter the resting current level in these Cs-filled postsynaptic neurons (data not shown).

Inhibition of EPSCs by HU-210 coincided with an increase in the PPR ratio (Fig. 1C). Both the PPR and CV (see METHODS) measured at corticostriatal synapses have been shown to increase following manipulations that lower the probability of glutamate release (Choi and Lovinger 1997a). As shown in Fig. 1D, application of 1 μM HU-210 led to significant increases in both PPR (136 ± 6%, P < 0.01) and CV (195 ± 21%, P < 0.01), suggesting a decrease in glutamate release probability following CB1 activation. Experiments using WIN 55,212–2 showed similar effects on PPR (161 ± 32%, P ≤ 0.05). The CB1 antagonist SR141716A prevented inhibition of glutamatergic synaptic transmission by 1 μM HU-210 (Fig. 2), confirming that effects on EPSC amplitude, PPR and CV were mediated through the CB1 receptor. Application of 2 μM SR141716A alone had no effect as compared with control experiments (EPSC = 102 ± 12%, P > 0.05, n = 4).

To further test the hypothesis that CB1 activation inhibits glutamate release in striatum, we analyzed quantal release events in the presence of strontium (Sr2+). It is known that replacing Ca2+ with Sr2+ decreases fast, synchronous release of neurotransmitter, while facilitating asynchronous, quantal release events for hundreds of milliseconds following afferent stimulation (Xu-Friedman and Regehr 2000). Application of 1 μM HU-210 decreased the synchronous evoked EPSC in six of seven cells (41 ± 6% baseline, data not shown), consistent with results in normal ACSF. Furthermore as shown in Fig. 3, 1 μM HU-210 caused a decrease in the frequency of asynchronous quantal release events (59 ± 6%, P < 0.05, paired t-test), with no change in amplitude distribution or rise-time kinetics (P > 0.05, Kolmogorov-Smirnov statistic). Spontaneous synaptic release events occurring between stimuli (see METHODS) were also analyzed, and were similarly decreased in frequency, but not amplitude (Fig. 3, C and D).

DISCUSSION

Our findings indicate that CB1 receptor activation inhibits excitatory synaptic responses in rat striatum and that this inhibition is caused by a decrease in glutamate release probability. Our findings in Sr2+ replacement experiments support this conclusion, and by demonstrating inhibition of spontaneous release events, these experiments suggest a mechanism downstream from presynaptic action potentials and Ca2+ channels. Thus while the observed effects of CB1 activation on evoked striatal glutamate release may involve modulation of presynaptic Ca2+ entry (Twitchell et al. 1997), a more direct
and Ca\textsuperscript{2+}-independent inhibition of the release process is also implicated by our results. It is interesting to note that studies of other glutamatergic synapses have also found cannabinoids to inhibit neurotransmitter release (Lévéne et al. 1998; Sañudo-Peña et al. 1999; Shen et al. 1996; Vaughan et al. 2000).

A parsimonious explanation for these findings is that the CB1 receptor is expressed presynaptically on cortical, or possibly thalamic, axon terminals in the dorsolateral striatum, where activation of this receptor leads to inhibition of glutamate release. Expression of CB1 receptors on afferent terminals in striatum has not been clearly shown. Herkenham et al. (1991) showed a near total loss in radioligand binding at the CB1 receptor following excitotoxic lesioning of the rat striatum. However their methods focused on medial striatal areas and may not have been sensitive to binding on cortical terminals in dorsolateral striatum, where the CB1 receptor is expressed more densely (Herkenham et al. 1991; Tsou et al. 1998). It should also be noted that the presynaptic effects seen in this study could be due to indirect effects of CB1 receptor activation, even if the CB1 receptors are not themselves on presynaptic terminals.

It is notable that the agonists used in this study acted with similar apparent potencies, whereas in binding experiments, HU-210 has been found to be \( \approx 100 \) times more potent than WIN 55,212–2 (Pertwee 1997). This discrepancy likely resulted from poorer solubility or availability of HU-210 within brain slices under the conditions of these experiments. The use of cytochrome c as a carrier agent may be an important factor in this regard.

In rat striatum in vivo, depolarizing conditions lead to increased detection of extracellular anandamide (Giufrida et al. 1999). Our results suggest that activity-dependent release of anandamide, either from presynaptic terminals or from the postsynaptic membrane, could serve as a feedback mechanism to inhibit further depolarizing glutamate input. This does not appear to occur during low-frequency synaptic activation, as SR141716A showed no effect on baseline transmission. It will be interesting to determine if CB1 receptors play a role in transmission or plasticity during higher frequency synaptic activation. CB1 activation has been found to inhibit GABAergic responses evoked by local stimulation of the neostriatum (Szabo et al. 1998). Thus CB1 receptor modulation of both excitatory and inhibitory input to medium spiny neurons may underlie dose-dependent, biphasic behavioral activities of cannabinoid agonists (Sañudo-Peña et al. 1999).

Cannabinoids have profound effects on movement in rodents, mediated through the striatum and its targets in the basal ganglia (Sañudo-Peña et al. 1999). The CB1 receptor is thus a potential pharmacological target in the treatment of extrapyramidal movement disorders. Regulation of corticostriatal glutamate release by cannabinoids suggests that further investigation of such a therapeutic strategy is warranted.

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