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

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Gerde et al. 1999. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85: 468–471, 2001. CB1 cannabinoid receptors are found in the striatum and their target nuclei. The CB1 receptor has been shown to inhibit neurotransmitter release in various brain areas, we investigated the effects of CB1 activation on glutamatergic synaptic transmission in the dorsolateral striatum of the rat. The CB1 receptor is highly expressed. We performed whole cell voltage-clamp experiments in striatal brain slices and applied the CB1 agonist HU-210 or WIN 55,212-2 during measurement of synaptic transmission. Excitatory postsynaptic currents (EPSCs), evoked by electrical stimulation of afferent fibers, were significantly reduced in a dose-dependent manner by CB1 agonist application. EPSC inhibition was accompanied by an increase in two separate indices of presynaptic release, the paired-pulse response ratio and the coefficient of variation, suggesting a decrease in neurotransmitter release. These effects were prevented by application of the CB1 antagonist SR141716A. When Sr2+ was substituted for Ca2+ in the extracellular solution, application of HU-210 (1 μM) significantly reduced the frequency, but not amplitude, of evoked, asynchronous quantal release events. Spontaneous release events were similarly reduced in 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 thus 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 biochemical, physiological, and behavioral data (Glass et al. 1997; Zimmer et al. 1999), suggest that modulation of striatal output is an important mechanism of cannabinoid actions. Anandamide, an endogenous ligand for the CB1 receptor (Devane et al. 1992), can be synthesized and released by depolarized rat striatal neurons both in culture (Di Marzo et al. 1994), and in vivo (Giuffrida et al. 1999), suggesting a neuromodulatory role for the CB1 receptor in the striatum.

While studies have shown effects of the CB1 receptor on efferent terminals of striatal projection neurons (Sañudo-Peña et al. 1999) and on inhibitory GABAergic synaptic transmission intrinsic to the striatum (Szabo et al. 1998), possible cannabinoid effects on excitatory cortical input to the striatum remain essentially unexplored. Signaling systems that modify corticostriatal synaptic transmission are likely to play a significant role in regulating striatal output, and may represent therapeutic targets for the treatment of Parkinson’s disease (Calabresi et al. 1996).

Activation of the CB1 receptor, which couples primarily to Gi/Go-type G proteins (Howlett et al. 1986; Pertwee 1997) and can modulate numerous ion channel conductances (Mu et al. 1999; Pertwee 1997; Twitchell et al. 1997), has been found to inhibit glutamate release in rat cerebellar slices (Lévênes et al. 1998), in hippocampal cultures (Shen et al. 1996) and in slices from periaqueductual gray (Vaughan et al. 2000). The present study examined the effects of the activation of cannabinoid CB1 receptors on glutamatergic synaptic transmission in the neostriatum, using electrophysiological techniques in the rat striatal brain slice. CB1 receptor activation was found to inhibit synaptic responses in striatal neurons by a presynaptic mechanism.

METHODS

Methods for striatal slice preparation and whole cell electrophysiology experiments were essentially the same as previously described (Choi and Lovinger 1997a,b). Coronal 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...
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 CsCl, 10 TEA chloride, 3–5 QX-314 (Br salt), 1.1 EGTA, 4 ATP (Mg²⁺ salt), and 0.3 GTP (Na salt), pH adjusted to 7.2 with CsOH. Neurons were voltage-clamped at −65 to −70 mV. Series resistance (<10 MΩ) was monitored continuously and was not compensated.

To evoke synaptic currents in normal ACSF, paired stimuli (50-ms interpulse interval) were delivered at 0.05 Hz through a bipolar electrode placed in the white matter overlying the lateral striatum. The range of stimulus parameters was 0.2–1.5 mA in amplitude and 20–200 μs in duration, and EPSC amplitudes ranged from 150 to 700 pA across all experiments. In Sr²⁺-substitution experiments, single stimuli were given at 0.1 Hz. Synaptic responses recorded during a 10-min period prior to drug application were used to calculate baseline values for peak EPSC amplitude, paired-pulse ratio (PPR), and coefficient of variation (CV). PPR was expressed as the ratio of EPSC amplitudes: EPSC 2/EPSC 1. CV was calculated as the coefficient of variation (CV). PPR was expressed as the ratio of EPSC amplitudes: EPSC 2/EPSC 1. CV was calculated as the coefficient of variation (CV).

Effects are shown as percentage of baseline ± SE. EPSC values are compared statistically to values measured after application of CB1 agonists using the Kolmogorov-Smirnov test. Effects of drug treatments were compared with baseline values using repeated measures, or compared with mean results from control experiments (see following text) using Student’s t-test assuming unequal variances, and P < 0.05 was considered statistically significant.
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 (Levénès 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 to 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 (Giuffrida 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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