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Acute Effects of the Cannabinoid Receptor Agonist WIN55212-2 on Dopamine Release in Rat: An *In Vivo* Electrochemical Study

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Abstract

The aim of this study was to investigate the effects of the cannabinoid receptor agonist, WIN55212-2, and the cannabinoid receptor antagonist, SR141716A, on dopamine (DA) release evoked by KCl (120 mM) microinjected into the striatum. The cannabinoid agonist WIN55212-2 (1 and 5 mg/kg, i.p.) dose-dependently attenuated DA release in the striatum, whereas the cannabinoid receptor antagonist SR141716A (3 mg/kg, i.p.) produced the opposite effect. SR141716A (3 mg/kg, i.p.) blocked the effects on DA release by WIN55212-2 (5 mg/kg, i.p.). Vehicle alone did not change DA release. These results suggest that cannabinoids modulate DA release in the striatum.

Key Words: dopamine, cannabinoid receptor, striatum

Introduction

The striatum is the major input nucleus of the basal ganglia. It plays an important role in movement control in both normal conditions and pathophysiological disorders such as Huntington's and Parkinson's Diseases (6). Cannabinoids such as WIN55212-2, CP55940, and Δ^9-tetrahydrocannabino profoundly influence motor behavior in rats (35) and affect motivational state and movement in humans (12). Two G protein-coupled receptors, CB_1 and CB_2, have been identified. CB_2 receptors are localized in the periphery in immune cells, whereas CB_1 receptors are distributed widely in the striatum and its target nuclei (13, 25, 34). Cannabinoids modulate [^{3}H]-DA release and adenylyl cyclase activity in the human neocortex (31). Many physiological, biochemical, and behavioral data (8) suggest that modulation of striatal output is an important mechanism of cannabinoid actions.

Cannabinoids inhibit the electrically evoked release of previously incorporated [^{3}H]dopamine (DA) from slices of rat striatum (5). Systemic administration of cannabinoids enhances DA release in the nucleus accumbens through enhanced firing of mesolimbic DAergic neurons (34). The CB_1 and CB_2 cannabinoid receptor agonists, WIN55212-2 and CP55940, and CB_1 cannabinoid receptor antagonist, SR141716A, have no effect on the electrically evoked DA release in the corpus striatum and nucleus accumbens (33). CB_1 receptors are located on dopaminergic nerve terminals and tonically activated by endocannabinoids (31). Moreover, Activation of CB_1 cannabinoid receptors affects the regulation of the sympathetic cardiovascular system, for example, intravenous administration of WIN55212-2 reduce blood pressure, and this effect is antagonized by SR141716A (21). Findings of behaviored studies show that, in unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats with Parkinson's Disease rats,

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cannabinoids modulate D_1 but not D_2 dopamine receptor-mediated effects (1). In contrast, in the reserpine-treated rat model of Parkinson's Disease, WIN55212-2 (0.1 and 0.3 mg/kg) decreases the action of D_2 receptor agonist quinpirole (0.1 mg/kg)-induced alleviation of akinesia, and this effect can be blocked by coadministration with SR141716A (3 mg/kg, i.p.) (18).

The aim of this study was to investigate the effects of the cannabinoid receptor agonist, WIN55212-2, and the cannabinoid receptor antagonist, SR141716A, on DA release evoked by KCl microinjected into the striatum. We used *in vivo* voltammetry to examine the effect of cannabinoids on the peak amplitude of DA, time for the signal to return to 80% of the maximal response (T_{80}), and the clearance rate (Tc) of DA release in the striatum.

Materials and Methods

All protocols were conducted following the National Institutes of Health Guidelines and handbook using the "Animals in Research" approved by the Institutional Animal Care and Use Committee (National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD).

Animals

Male Sprague Dawley rats (Charles River Labs, Raleigh, NC, USA), weighing 450 - 550g, were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic apparatus on top of a heating pad to maintain the body temperature at 37°C throughout the experiment. A portion of the skull was removed completely to expose the brain anterior to the bregma (+ 3.0 mm anterior and ±3.5 mm lateral). Electrodes were glued to a glass micropipette (10-20 μm O.D.) with tip separation of about 150-250 μm using sticky wax (Kerr Brand, Emeryville, CA, USA). The pipette contained 120 mM KCl to stimulate DA release. The electrode-pipette assembly was lowered slowly into the striatum according to the atlas of Paxinos and Watson (23) (AP +1.5 mm, L ± 2.0 mm, DV -3.0 to -7.5 mm with respect to the bregma and the cortical surface). The electrode was placed randomly on the left or right side. An Ag^+/AgCl reference electrode was placed just below the cortical surface anterior to the microelectrode and cemented to the skull with dental cement. DA release was induced by pressure ejection of KCl (120 mM, 500 ± 50 nl) using chronoamperometry through the implanted pipette at 3-20 psi for 2-6 s delivered by an IVEC-10 computer controlled system (PPS-2, PPM-2, Medical Systems, Inc., Greenvale, NY, USA). The actual volume ejected was determined by a calibrated reticule on the eyepiece of a dissecting microscope. At least two releases were measured in each location with a 15 min delay between release attempts. Three parameters of release were examined: (i) peak amplitude, the concentration of DA released extracellularly; (ii) T_{80}, the time taken for the signal to return to 80% of the maximum response; and (iii) clearance rate (Tc, in μM/s), defined by the change in DA concentration between the T_{20} and T_{60} time points. At the end of the experiment, the rats were euthanized with an overdose of urethane.

Electrochemistry

Double carbon fiber electrodes (100 μm length × 30 μm O.D.) were coated with Nafion using a high-temperature coating procedure (7). All electrodes were calibrated using 2-10 μM increments of DA before each experiment and all electrodes were both linear ($r^2 \geq 0.997$) and selective (≥ 500:1) for DA *vs.* ascorbate. Extracellular changes in DA concentration were expressed quantitatively based on the preexperiment electrode calibrated curves (7). DA release determinations were made by the IVEC-10 system described above. Experimental data were collected at a 5 Hz sampling frequency and averaged over 1 s to improve the signal-to-noise ratio with an applied potential of 0.55 V *vs.* the Ag^+/AgCl reference. The current was integrated over the last 80 ms of each data set. The electrode was brought back to resting potential (0.00 V), and the resulting reduction in current was analyzed as before. The redox ratio for DA was between 0.3 and 0.6 under these experimental conditions.

Chemicals

The drugs were obtained from WIN55212-2 from Tocris Cookson (Ballwin, MO, USA), and SR141716A from the National Institute on Drug Abuse drug supply system. WIN55212-2 was prepared at concentrations of 1 mg/ml and 5 mg/ml, and SR14176A was prepared at a concentration of 3 mg/ml in vehicle (70% NaCl, 10% Tween 80, and 20% DMSO).

Statistical Analysis

The analysis of Group data were presented as the mean ± SEM. Drug effects on the amplitude of DA release, T_{80}, and Tc were analyzed using Student's *t*-test and ANOVA. $*P < 0.05$ or $**P < 0.01$ were considered significant.

Results

The effects of local pressure application of KCl on DA release, T_{80}, and Tc at different depths below the surface (from -3 to -7.5 mm, one step = 0.5 mm) of the striatum are shown in Fig. 1. In 20 experiments,

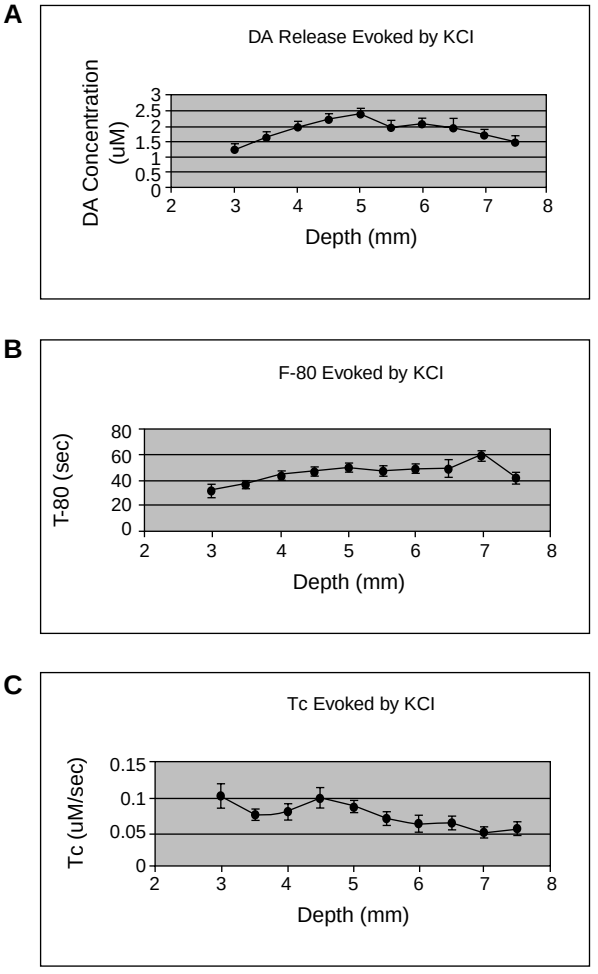

Fig. 1. The effects of local injection of KCl on electrochemical parameters in different depths of the striatum. A, Response of DA release. B, Response of T_{80}. C, Response of Tc.

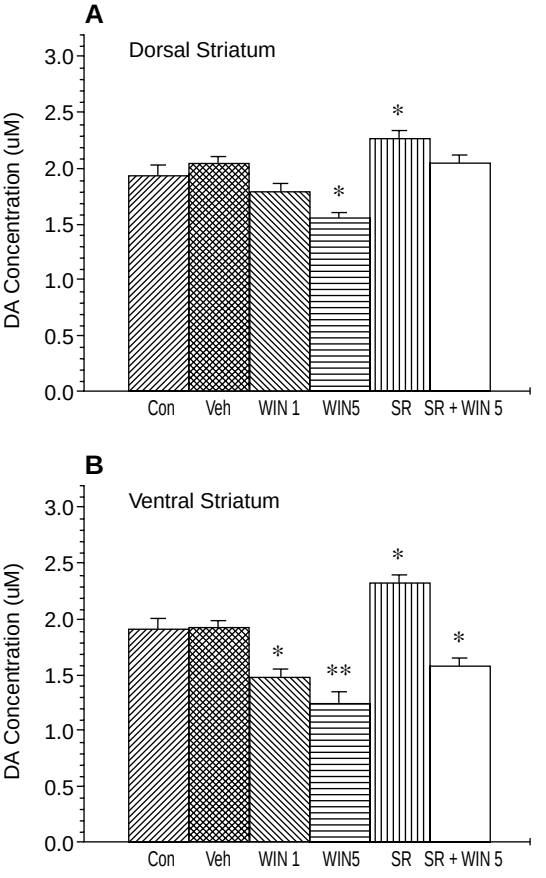

Fig. 2. Effects of chemical administration on DA release compared with the control (Con) in the striatum. A, In the dorsal striatum, DA release was unchanged by vehicle (Veh) (n = 6; $P > 0.05$), nonsignificantly decreased by WIN55212-2 (1 mg/kg, WIN 1) (n = 6, $P > 0.05$), decreased by WIN55212-2 (5 mg/kg, WIN 5) (n = 6, $*P < 0.05$), and increased by SR141716A (3 mg/kg, SR) (n = 6, $*P < 0.05$). In contrast, SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) did not change DA release (n = 6, $P > 0.05$). B, In the ventral striatum, DA release was unchanged by vehicle (Veh) (n = 6; $P > 0.05$), decreased by WIN55212-2 (1 mg/kg, WIN 1) (n = 6, $*P < 0.05$) and WIN55212-2 (5 mg/kg, WIN 5) (n = 6, $**P < 0.01$), and increased by SR141716A (3 mg/kg, SR) (n = 6, $*P < 0.05$). In contrast, SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) decreased DA release (n = 6, $*P < 0.05$). Values are expressed as mean ± SEM. $*P < 0.05$ and $**P < 0.01$ compared with the control group.

after microinjection of KCl (120 mM, 500 ± 50 nl) into the striatum, DA was released at concentrations of 1.2-2.5 μM (Fig. 1A), T_{80} was 32-60 s (Fig. 1B), and Tc was 0.05-0.11 μM/s (Fig. 1C). Based on the peak DA released from different depths, we divided the striatum into a dorsal part (from -3 to -5.5 mm) and a ventral part (from -6.0 to -7.5 mm).

Effect on DA

As shown in Fig. 2A, in the dorsal striatum, vehicle and WIN55212-2 (1 mg/kg) caused different changes in DA release. WIN55212-2 (5 mg/kg) significantly decreased DA release, whereas SR141716A (3 mg/kg) significantly increased DA release. SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) did not change DA release. In the ventral striatum, vehicle did not change DA release. WIN55212-2 at the low (1 mg/kg) and higher (5 mg/kg) concentration significantly decreased DA release. In contrast, SR141716A (3 mg/kg) significantly increased DA release. SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) significantly decreased DA release (Fig. 2B).

Effect on T_{80}

As shown in Fig. 3A, in the dorsal striatum, vehicle

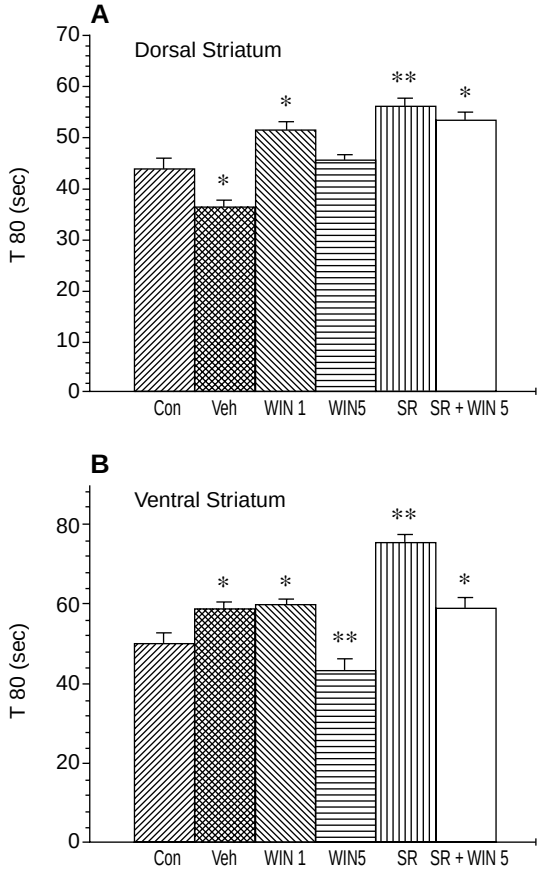

Fig. 3. Effects of chemical administration on T_{80} compared with the control (Con) in the striatum. A, In the dorsal striatum, T_{80} was decreased by vehicle (Veh) (n = 5; $*P < 0.05$), increased by WIN55212-2 (1 mg/kg, WIN 1) (n = 5, $*P < 0.05$), and not changed by WIN55212-2 (5 mg/kg, WIN 5) (n = 5, $P > 0.05$). In contrast, SR141716A (3 mg/kg, SR) increased T_{80} (n = 5, $**P < 0.01$), and SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) increased T_{80} (n = 5, $*P < 0.05$). B, In the ventral striatum, T_{80} was increased by vehicle (Veh) (n = 5; $*P < 0.05$) and WIN55212-2 (1 mg/kg, WIN 1) (n = 5, $*P < 0.05$) and decreased by WIN55212-2 (5 mg/kg, WIN 5) (n = 5, $**P < 0.01$). In contrast, SR141716A (3 mg/kg, SR) increased T_{80} (n = 5, $**P < 0.01$), and SR141716A (3 mg/kg) 15 min before WIN55212-2 (5 mg/kg) increased T_{80} (n = 5, $*P < 0.05$). Values are expressed as mean ± SEM. $*P < 0.05$ and $**P < 0.01$, compared with the control group.

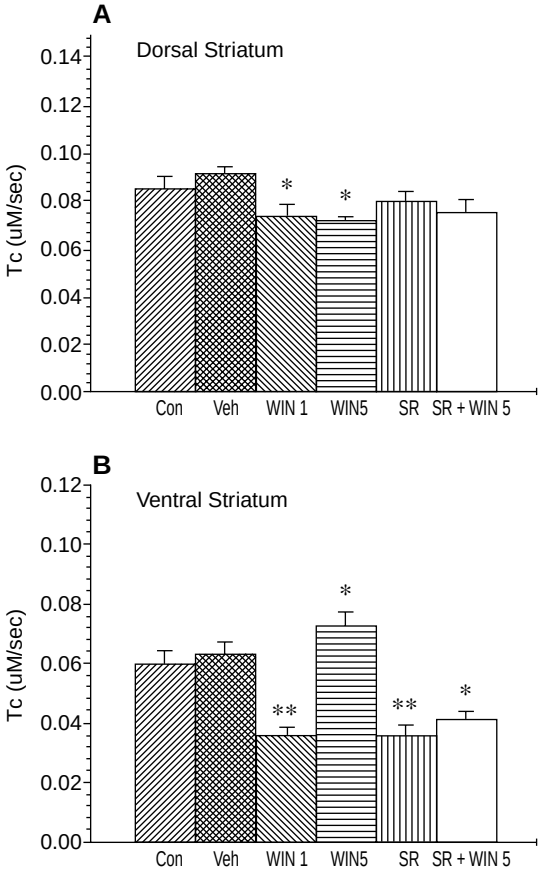

Fig. 4. Effects of chemical administration on Tc compared with the control (Con) in the striatum. A, In the dorsal striatum, Tc was unchanged by vehicle (Veh) (n = 6; $P > 0.05$), decreased significantly by WIN55212-2 (1 mg/kg, WIN 1) (n = 6, $*P < 0.05$), decreased by WIN55212-2 (5 mg/kg, WIN 5) (n = 6, $*P < 0.05$), and unchanged by SR141716A (3 mg/kg, SR) (n = 6, $P > 0.05$). SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) did not change Tc (n = 6, $P > 0.05$). B, In the ventral striatum, Tc was unchanged by vehicle (Veh) (n = 6; $P > 0.05$) and decreased by WIN55212-2 (1 mg/kg, WIN 1) (n = 6, $**P < 0.01$). In contrast, Tc was increased by WIN55212-2 (5 mg/kg, WIN 5) (n = 6, $*P < 0.05$) and decreased by SR141716A (3 mg/kg) (n = 5, $**P < 0.01$). SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) decreased Tc (n = 6, $*P < 0.05$). Values are expressed as mean ± SEM. $*P < 0.05$ and $**P < 0.01$ compared with the control group.

decreased T_{80}, WIN55212-2 at 1 mg/kg increased T_{80}, and WIN55212-2 at 5 mg/kg did not change T_{80}. SR141716A (3 mg/kg) alone or SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) significantly increased T_{80}. In the ventral striatum, vehicle or WIN55212-2 (1 mg/kg) increased T_{80}, but WIN55212-2 (5 mg/kg) decreased T_{80}. SR141716A (3 mg/kg) alone, and SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) increased T_{80} (Fig. 3B).

Effect on Tc

As shown in Fig. 4A, in the dorsal striatum, vehicle increased Tc slightly, and WIN55212-2 (1 mg/kg) and WIN55212-2 (5 mg/kg) decreased Tc. SR141716A (3 mg/kg) alone and SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) decreased Tc slightly. In the ventral striatum, vehicle increased Tc slightly, and WIN55212-2 (5 mg/kg)

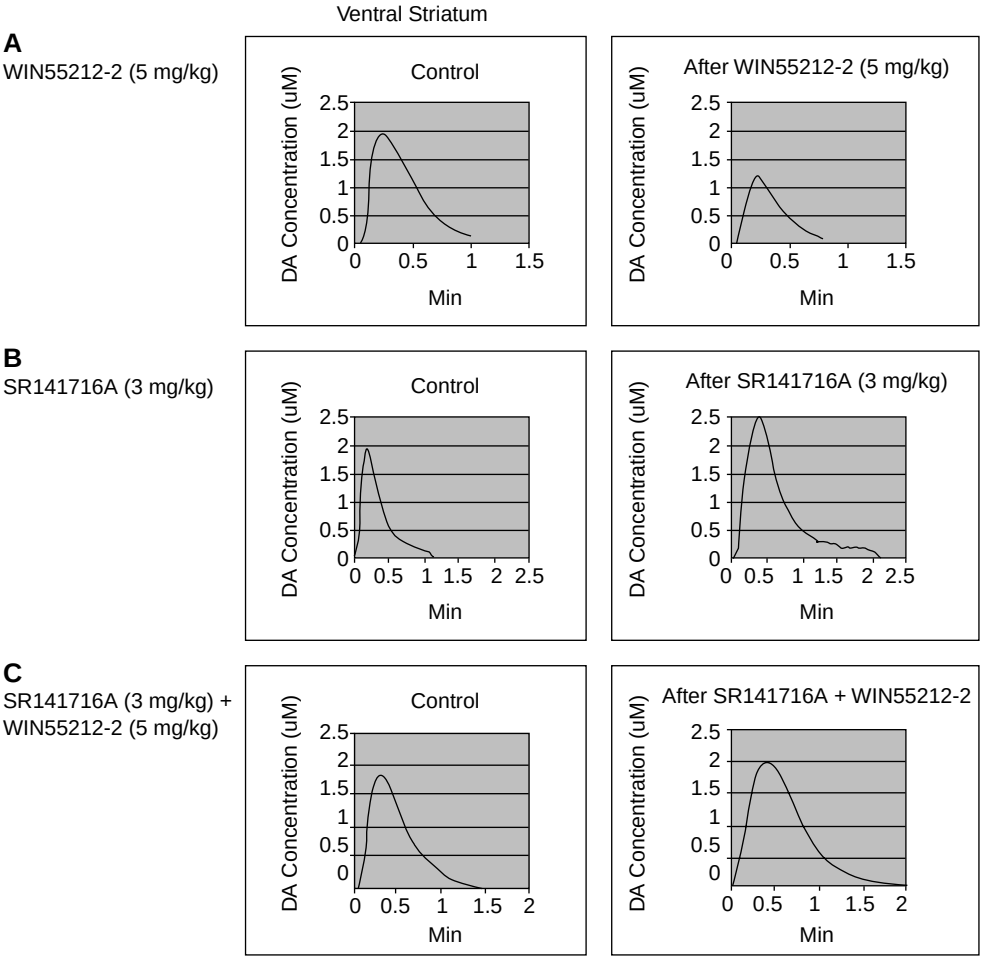

Fig. 5. Representative electrochemical profiles of dopamine (DA) release evoked by KCl from the ventral striatum of the control and after W55212-2 and SR141716A administrations. A, Effect of W55212-2 (5 mg/kg) on DA release. B, Effect of SR141716A (3 mg/kg) on DA release. C, Effect of administration of SR141716A (3 mg/kg) and WIN55212-2 (5 mg/kg) on DA release. Note that the effect of WIN55212 on DA was blocked by SR141716A.

increased Tc. WIN55212-2 (1 mg/kg), SR141716A (3 mg/kg) alone, and SR141716A (3 mg/kg) given 15 min before WIN55212-2 (5 mg/kg) significantly decreased Tc (Fig. 4B).

Interaction of WIN55212-2 and SR141716A

In seven experiments, in the ventral striatum, the effects of WIN55212-2 were blocked by SR141716A (Fig. 5). Microinjection of KCl (120 mM, 500 ± 50 nl) produced DA release (Fig. 5, left panel). Fifteen min after WIN55212-2 (5 mg/kg, i.p.), microinjection of KCl again produced small increase DA release (Fig. 5A, right panel). Fifteen min after SR141716A (3 mg/kg, i.p.), microinjection of KCl again produced large increase DA release (Fig. 5B, right panel). Fifteen min after WIN55212-2 (5 mg/kg, i.p.) and SR141716A (3 mg/kg, i.p.), microinjection of KCl again produced increase DA release (Fig. 5C, right panel) which is equal to microinjection of KCl alone.

Discussion

Activation by the cannabinoid receptor agonist WIN55212-2 inhibited DA release in the striatum, whereas activation by the cannabinoid receptor antagonist SR141716A increased DA release. Specifically, inhibition of DA release by WIN55212-2 was blocked by SR141716A. These results suggest that cannabinoids may participate in modulating DA release in the striatum.

To evoke the DA release at the striatum area, we activated the DA neurons by KCl injection to increase the K^+ ions extracellularly. The increase in K^+ in the extracellular compartment would reduce the outward K^+ ions current so that the DA neurons may be depolarized and excited to release the DA. In this study we found that WIN55212-2, a cannabinoid

agonist, and SR141716A, a cannabinoid receptor antagonist, produced opposite actions. This is consistent with the findings that WIN55212-2 (1 mg/kg) inhibits the increased firing rate of the substantia nigra (SNr) neurons induced by bicuculline stimulation of the subthalamic nucleus and that such effect is antagonized by SR141716A (1 mg/kg) (27). Together with the findings that WIN55212-2 increases the spontaneous firing rate of neurons in the substantia nigra pars reticulata (SNpr) (19) and the presynaptic localization of the receptor (10), thest results suggest that cannabinoids exert their effect by modulating DA release or reuptake in the striatum. Our data provide evidence that cannabinoids may participate in modulating the DA release in the striatum.

Autoradiography showed great heterogeneity of binding in patterns of labeling that closely conform to cytoarchitectural and functional domains (11). Very dense ^{3}H-CP55940 binding is localized to the basal ganglia (lateral caudate-putamen, globus pallidus, substantia nigra pars reticulata, entopeduncular nucleus), cerebellar molecular layer, innermost layers of the olfactory bulb, and portions of the hippocampal formation (CA3 and dentate gyrus molecular layer). Moderately dense binding is found throughout the remaining forebrain. Sparse binding characterizes the brain stem and spinal cord. It suggests that cannabinoid receptor mediates physiological and behavioral effects of natural and synthetic cannabinoids, because it is strongly coupled to guanine nucleotide regulatory proteins and is discretely localized to cortical, basal ganglia, and cerebellar structures involved with cognition and movement.

In our system, administration of SR141716A enhanced DA release in the striatum. This effect is similar to the observation that cannabinoid antagonists enhance neurotransmitter release (15, 22). Prior administration of SR141716A blocked the effect of WIN55212-2 on the inhibition of DA release in the striatum. These data show a competitive interaction between SR141716A and WIN55212-2, in agreement with the competitive inhibition of CP55940 shown in binding experiments (26). The effects of antagonists may result from the blockade of endogenous cannabinoid agonists. This is consistent with the finding that administration of SR141716A increases the spontaneous activity of dopamine A_9 cells, which inhibits GABA release from striatal terminals (9). SR141716A, by its antagonizing actions, increases GABA release, which decreases GABAergic neuronal activity in the SNpr. SR141716A leads to disinhibition of SNpr target cells (9). Following SR141716A application, CP55940 did not increase the SNpr firing rate above baseline, showing reversal of the SR141716A effect (35).

Activation of the CB_1 receptor, which primarily couples to G protein (13, 17, 25), modulates numerous ion channel conductance (36) and inhibits adenylate cyclase (14). The latter inhibits glutamate release in rat cerebellar slices (16), in periaqueductal gray slices (37) and in hippocampal cultures (29). For example, activation of CB1 receptors inhibits synaptic transmission through the activation of K^+ channels (17) and inhibition of voltage-gated Ca^{2+} channels (28). G protein activation by CB_1 and CB_2 receptors modulates the Krox-24 (Zenk) induction and mitogen-activated protein kinase signaling pathway (4).

Activation of CB1 cannabinoid receptors leads to presynaptic inhibition of the GABAergic neurotransmission between striatonigral axons and SNpr neurons (38). Inhibition of the inhibitory postsynaptic currents by WIN55212-2 and CP55940, and blockade of the effect by SR141716A, strongly suggests that CB_1 cannabinoid receptors are involved in the inhibition of GABAergic neurotransmission (24, 38). This result consists with the notion that cannabinoids inhibit cholinergic, glutamatergic, noradrenergic, and serotonergic neurotransmission in many brain regions (29, 32). Moreover, CP55940 and anandamide caused significant reductions in the release of DA after electrical stimulation of [^{3}H]dopamine-prelabelied striatal slices, which were antagonised by SR14176. The CB_1 receptor may influence DA release in the striatum suggests that cannabinoids play a modulatory role in dopaminergic neuronal pathways (5).

Unilateral application of WIN55212-2 produces contralateral circling behavior in a dose-effect manner, whereas SR141716A dose-dependently reduces WIN55212-2-induced turning in rats with 6-OHDA lesions in the striatum (30). Recent behavioral studies in the unilateral 6-OHDA-lesioned rat model of Parkinson's Disease suggest that cannabinoids modulate the D_1 dopamine receptor-mediated effects (1). The cannabinoid agonists of WIN55212-2 (2.5 mg/kg) and CP55940 (0.1 mg/kg) markedly attenuate contralateral rotation induced by the dopamine D_1 agonist SKF 38393 (1.5 mg/kg), but not by the dopamine D_2 agonist quinpirole (0.1 mg/kg) (1). In contrast, a reserpine-treated rat model of Parkinson's Disease suggests that WIN55212-2 (0.1 and 0.3 mg/kg) reduces the D_2 receptor agonist quinpirole (0.1 mg/kg)-induced alleviation of akinesia and shows that this effect is blocked by coadministration with SR141716A (3 mg/kg, i.p.) (18). These results contrast with previous reports based on the unilateral model of Parkinson's Disease, in which the cannabinoid receptor agonist CP55940 has no effect on D_2 receptor agonist-mediated contralateral rotation in the 6-OHDA-lesioned rat (1). The effects of D_2 stimulation of Parkinson's Disease symptoms are mediated through the striatal outputs, which influence basal ganglia outputs indirectly. These results suggest that cannabinoids modulate neurotransmission in the pathway linking the striatum indirectly to the basal

ganglia outputs through the lateral globus pallidus and the subthalamic nucleus (18).

The appearance of the highest density of cannabinoid receptors in the striatum, SNpr, and nucleus accumbens strongly suggest an association with DA receptors. The basal ganglia have been implicated in the cataleptic action of cannabinoids. The cannabinoid receptor antagonist SR141716A (0.5 and 2.5 mg/kg) reduces catalepsy elicited by the cannabinoid receptor agonist CP55940 (0.5 mg/kg) (2). The cannabinoid effects include decreases in spontaneous locomotor activity and stereotypic behaviors such as rearing and grooming (20). Intranigra injections of GABA agonists increase locomotion and behaviors such as sniffing (3). That is, cannabinoids affect the striatum and the striatum may be the site of action by cannabinoids in mediating spontaneous locomotion and stereotypic behaviors.

Cannabinoids have an antinociceptive action and anticonvulsant effect, which appear to be mediated by the brain cannabinoid receptor. For example, injection of GABA or muscimol into the substantia nigra inhibits SNpr function associated with antinociceptive activity (3). The anticonvulsant effect is generally associated with drug actions in the SNpr by decreasing SNpr neuronal activity (39). We observed that WIN55212-2 decreases DA release in striatum cells, an effect that is similar to the reported actions of muscimol and GABA on SNpr activity (40).

Local injection of KCl, but not vehicle, caused DA release in the striatum. This demonstrates the effectiveness of our technique using local pressure injection from a glass capillary electrode assembled with a DA electrode. This technique suggests that these effects contribute to actions at or near the recording site rather than from a distant site.

The major findings of this study are that the cannabinoid receptor agonist WIN55212-2 attenuates DA release in the striatum, whereas the cannabinoid receptor antagonist SR141716A increases DA release. In addition, the effects of WIN55212-2 are blocked by SR141716A. These results suggest that WIN55212-2 modulates DA release in the striatum.

Acknowledgments

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