Effects of Exogenous and Endogenous Cannabinoids on GABAergic Neurotransmission between the Caudate-Putamen and the Globus Pallidus in the Mouse

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ABSTRACT

Globus pallidus neurons receive GABAergic input from the caudate-putamen via the striatopallidal pathway. Anatomical studies indicate that many CB1 cannabinoid receptors are localized on terminals of striatopallidal axons. Accordingly, the hypothesis of the present work was that activation of CB1 receptors presynaptically inhibits neurotransmission between striatopallidal axons and globus pallidus neurons. In sagittal mouse brain slices, striatopallidal axons were electrically stimulated in the caudate-putamen, and the resulting GABAergic inhibitory postsynaptic currents (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-c]-cannabinoid receptor agonists (IPSCs) were recorded in globus pallidus neurons. The synthetic cannabinoid receptor agonists R(+)-(2,3-dihydro-5-

The present work focused on the neuronal connection between the caudate putamen and the globus pallidus (also called external or lateral globus pallidus). Globus pallidus neurons receive strong GABAergic input from medium spiny neurons of the caudate-putamen (striatopallidal projection neurons; Gerfen, 2004). The concentration of CB1 receptor protein in the globus pallidus is very high (Herkenham et al., 1991b; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998). Two kinds of observations support the idea that the majority of CB1 receptors in the globus pallidus is localized

ABBREVIATIONS: ACSC, artificial cerebrospinal fluid; IPSC, inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; DNQX, 6,7-dinitroquinoxaline-2,3-dione; AP5, dl-2-amino-5-phosphonopentanoic acid; DSI, depolarization-induced suppression of inhibition; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CNB-caged GABA, γ-aminobutyric acid-β-carboxy-2-nitrobenzyl-ester; ROI, region of interest; PRE, initial reference value determined before drug application; NO-711, 1-(2-[[((dimethylamino)ethoxy)methyl]oxymethy]phenoxy)ethanol; WIN55212-2, R(+)-(2,3-dihydro-5-methyl-3-[[morpholinyl][methyl] pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)-methanone mesylate; DMSO, dimethylsulfoxide; SOL, solvent.

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on axon terminals of striatopallidal GABAergic neurons. First, many, if not all, medium spiny neurons synthesize CB1 receptor mRNA (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Hohmann and Herkenham, 2000). More specifically, all striatopallidal neurons (which synthesize the neurochemical marker preproenkephalin mRNA) synthesize CB1 receptor mRNA (Hohmann and Herkenham, 2000). Second, the density of CB1 receptors in the globus pallidus decreases strongly if medium spiny neurons in the caudate-putamen are experimentally damaged (Herkenham et al., 1991a).

The hypothesis of the present work was that activation of CB1 receptors in terminals of striatopallidal axons modulates GABAergic synaptic transmission between these axons and globus pallidus neurons. For testing the hypothesis, we carried out a comprehensive electrophysiological analysis of striatopallidal neurotransmission in mouse brain slices. In addition to studying effects of synthetic exogenous cannabinoid receptor agonists, we also searched for synaptic modulation by endocannabinoids.

Globus pallidus neurons receive GABAergic input not only from the caudate-putamen but also from neighboring globus pallidus neurons. For studying the effect of cannabinoids on striatopallidal neurotransmission, we selectively activated the striatopallidal pathway by stimulation in the caudate-putamen and recorded the resulting GABAergic synaptic currents in globus pallidus neurons.

Materials and Methods

The experiments conformed to the rules of the German law regulating the use of animals in biomedical research. All efforts were made to minimize both the suffering and the number of animals used. The methods were similar to those described previously (Szabo et al., 2004; Freiman and Szabo, 2005).

Brain Slices. Ten- to 18-day-old (for electrophysiological recordings) or 31- to 35-day-old (for calcium imaging) NMRI mice were anesthetized with isoflurane and decapitated. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition: 126 mM NaCl, 1.2 mM NaH2PO4, 3 mM KCl, 5 mM MgCl2, 1 mM CaCl2, 26 mM NaHCO3, 20 mM glucose, and 4 mM sodium lactate, pH 7.3 to 7.4 (after the solution was gassed with 95% O2/5% CO2). Oblique-sagittal slices (300-μm thick) including the globus pallidus and the caudate-putamen were cut at an angle of 20° to the midline. The slices were stored ond, the density of CB1 receptors in the globus pallidus neurons.

Recording of Inhibitory Postsynaptic Currents (IPSCs), Miniature IPSCs (mIPSCs), and Muscimol-Evoked Currents. IPSCs, mIPSCs, and muscimol-evoked currents in globus pallidus neurons were recorded in whole-cell configuration at a holding potential of -60 mV with pipettes containing 142 mM CsCl, 1 mM MgCl2, 10 mM HEPES, 4 mM ATP-Na2, and 2 mM N-ethyl-lidocaine chloride, pH 7.4. The superfusion ACSF contained DNQX (10−5 M) and AP5 (2.5 × 10−5 M) to suppress fast glutamatergic neurotransmission. IPSCs were elicited every 2 to 15 s with a bipolar platinum/iridium electrode positioned in the caudate-putamen. Single rectangular electrical pulses (10- to 100-μs pulse width; 1- to 3-mA pulse amplitude) were delivered by an isolated stimulator. Usually, 10 IPSCs were averaged. Muscimol-evoked currents were evoked every 60 s by pressure ejection of muscimol (10−3 M) from a pipette positioned about 100 μm above the surface of the slice. Pressure pulses (100-ms pulse width; 35- to 70-kPa amplitude) were delivered by a Picopump 820 (World Precision Instruments, Berlin).

A

B

C

D

Fig. 1. Properties of neurons in the globus pallidus. A, infrared video microscopic image of neurons before patch clamping. B, firing of a neuron recorded in the cell-attached mode before breaking into the cell (voltage clamp, holding potential = 0 mV). C, firing of a neuron recorded in the whole-cell mode immediately after breaking into the cell (current clamp, holding current = 0 pA). D, response of a neuron to hyperpolarizing current injections. At high negative membrane potentials, slowly developing depolarizations appear (arrowhead). After the hyperpolarizing current injections, rebound action potentials can be observed (arrow). The recordings in B, C, and D are from the same neuron.
Germany). Five muscimol-evoked currents were averaged for further evaluation. mIPSCs were recorded in the presence of tetrodotoxin (3 × 10⁻⁷ M) in 60-s periods and identified and analyzed using the MiniAnalysis software (version 5.2.6; Synaptosoft, Decatur, GA). For studying depolarization-induced suppression of inhibition (DSI), the pipette solution contained 147 mM CsCl, 1 mM MgCl₂, 10 mM HEPES, 1 mM EGTA, 4 mM ATP-Na₂, 0.4 mM GTP-Na, and 2 mM N-ethyl-lidocaine chloride, pH 7.4.

**Flash Photolysis of Caged GABA.** Globus pallidus neurons were patched with pipettes containing the CsCl-based intracellular solution used to record IPSCs. The slices were superfused with ACSF containing γ-aminobutyric acid-o-carboxy-2-nitrobenzyl-ester (CNB-caged GABA) (8 × 10⁻⁵ M). An ultraviolet flash light source was connected to the microscope via a quartz light guide and a special condenser (TILL Photonics, Gräfelfing, Germany). Flashes (illuminated spot size, 50 × 50 μm) were applied every 60 s.

**Fluorescence Measurement of Calcium Concentrations in Globus Pallidus Neurons.** The patch pipette contained the same intracellular solution which was used to study DSI and, in addition, the low-affinity calcium indicator (K_d for calcium, 2 × 10⁻⁴ M) Oregon Green 488 BAPTA-5N (final concentration in the pipette, 2 × 10⁻⁴ M).

Fluorescence intensity in globus pallidus neurons was determined with an imaging system consisting of Polychrome IV monochromatic light source, a cooled IMAGO VGA CCD camera, and TILLvision imaging software (all components from TILL Photonics). With the regularly used 40 x objective lens and at 2-fold binning, the camera had a pixel size of 0.5 μm. For measuring Oregon Green fluorescence, the excitation wave length of the monochromatic light source was adjusted to 495 nm, and a dichroic filter of 505DRLP and a bandpass filter were used. The excitation wavelength and the fluorescence filter set were also identical. Eight-fold binning was used; this resulted in a camera pixel size of 2 μm.

At each measurement period, 40 fluorescence images of the globus pallidus were recorded at 25 Hz (interimage interval, 40 ms) (see Fig. 9). The striatopallidal axons were stimulated in the caudate-putamen after the fifth image. Electrical stimulation caused an inhomogeneous increase in fluorescence in the globus pallidus, most likely because some axons were not properly loaded with the fluorescent dye. We decided to evaluate fluorescence in ROIs, in which the electrical stimulation caused the strongest fluorescence increases. Fluorescence values were corrected for background fluorescence. For further evaluation, ratios between stimulation-evoked fluorescence changes (ΔF) and baseline fluorescence measured immediately before stimulation (F₀) were calculated (ΔF/F₀ ratios).

**Protocols and Statistics.** Electrophysiological recordings started 20 min after establishment of the whole-cell configuration. Fluorescence recordings started 20 min after the beginning of superfusion in the bath chamber. Zero time in the figures is the time when recording began. Solvent and drug superfusion is indicated in the figures. When the cannabinoid antagonist rimonabant was applied in the DSI experiments, its superfusion started at least 15 min before the DSI protocol. Values of parameters during superfusion with solvent or drugs were expressed as percentages of the initial reference values (PRE; the PRE period is indicated in the figures).

Means ± S.E.M. are given throughout. Nonparametric statistical tests were used to identify significant differences. The two-tailed Mann-Whitney test was used for comparisons between groups (drug versus solvent); significant differences are indicated by an asterisk (*). The two-tailed Wilcoxon signed rank test was used for comparisons within groups (drug versus PRE); significant differences are indicated by + and #. p < 0.05 was taken as the limit of statistical significance, and only this level is indicated, even if p was < 0.01 or < 0.001.

**Drugs.** Drugs were obtained from the following sources. N-ethyl-lidocaine chloride (QX-314) was from Alamone Labs (Jerusalem, Israel); Oregon Green 488 BAPTA-5N hexapotassium salt, Oregon Green 488 BAPTA-1 dextran (mol. wt. 10,000), and CNB-caged GABA were from Invitrogen (Leiden, The Netherlands); rimonabant (previously called SR141716A) was from Sanofi-Aventis (Chilly-Mazarin, France); NO-711 was from Sigma Chemie (Deisenhofen, Germany); and HU210, CP55940, 6,7-dinitroquinoxaline-2,3-dione (DNQX), DL-2-amino-5-phosphonopentanoic acid (AP5), quinpirole HCl, WIN55212-2, and tetrodotoxin were from Tocris Cookson (Bristol, UK).

The cannabinoid ligands WIN55212-2, CP55940, HU210, and rimonabant were dissolved in dimethylsulfoxide (DMSO). Stock solutions were stored at –20 °C. Further dilutions were made with superfusion buffer; the final concentration of DMSO in the superfusion fluid was 1 ml⁻¹. Control solutions always contained the appropriate concentration of DMSO.

**Results**

**Basic Properties of Globus Pallidus Neurons.** Neurons in the globus pallidus were characterized using pipettes containing a potassium gluconate-based solution. Most of the neurons were spontaneously active. Thus, 14 of 16 neurons were firing action potentials in the cell-attached configuration; the mean firing rate was 6 ± 1 Hz (n = 14; Fig. 1B shows a spontaneously active neuron). Immediately after establishment of the whole-cell configuration, 13 of the 16 neurons were firing spontaneously; the mean firing rate was 11 ± 1 Hz (n = 13) (Fig. 1C). In 11 of the 16 neurons, hyperpolarizing current injections elicited slowly developing depolarizations, and rebound action potentials appeared after the hyperpolarizing currents (Fig. 1D). The depolarizations were...
most probably mediated by the time- and voltage-dependent inward rectifier $I_h$. Cell resistance and cell membrane capacitance were $512 \pm 60 \, \text{M\Omega}$ ($n = 19$) and $29 \pm 3 \, \text{pF}$ ($n = 19$), respectively. The properties of our neurons resemble the properties determined previously by Cooper and Stanford (2000). GABAergic striatopallidal neurotransmission was studied in all globus pallidus neurons, irrespectively of their electrophysiological properties.

**Inhibitory Neurotransmission between the Caudate-Putamen and Globus Pallidus.** Electrical stimulation with single pulses in the caudate-putamen in the presence of ionotropic glutamate receptor antagonists elicited typical GABA$_A$ receptor-mediated IPSCs in globus pallidus neurons (Fig. 2). The amplitude of IPSCs was $274 \pm 23 \, \text{pA}$ ($n = 92$). The latency was $9.4 \pm 1.2 \, \text{ms}$ ($n = 92$); this long latency is due to the long distance between the stimulation electrode in the caudate-putamen and the site of recording of IPSCs in the globus pallidus. The GABA$_A$ receptor antagonist bicuculline ($2 \times 10^{-5} \, \text{M}$) abolished the IPSCs (Fig. 2A). The reversal potential was very near to the calculated chloride equilibrium potential (Fig. 2B). Finally, we tested whether a known modulator of striatopallidal neurotransmission caused the expected change in our preparation. The dopamine D$_2/D_3$-receptor agonist quinpirole ($10^{-5} \, \text{M}$) markedly inhibited striatopallidal neurotransmission (Fig. 2C) like dopamine did similarly in a previous study by activating D$_2$ receptors (Cooper and Stanford, 2001).

In control experiments in which solvent (SOL) was superfused, IPSCs slightly decreased (see SOL groups in Figs. 3 and 4). The decrease may be due to the high concentration of DMSO ($1 \, \text{ml l}^{-1}$) in the control solution; this concentration of DMSO was, however, necessary to keep cannabinoids in solution.

**Activation of CB$_1$ Cannabinoid Receptors Inhibits Neurotransmission.** The mixed CB$_1$/CB$_2$ cannabinoid receptor agonist WIN55212-2 ($3 \times 10^{-7}$ and $10^{-5} \, \text{M}$) was superfused for 15 min (Fig. 3A). At the lower concentration ($3 \times 10^{-7} \, \text{M}$), WIN55212-2 had a small effect which, however, was not significant. At the higher concentration ($10^{-5} \, \text{M}$), WIN55212-2 lowered the amplitude of IPSCs by 64% (corrected for the decrease observed in the solvent group). CP55940 ($10^{-5} \, \text{M}$), another mixed CB$_1$/CB$_2$ cannabinoid receptor agonist, also inhibited the IPSCs; the inhibition was 35% (corrected for the decrease in the solvent group) (Fig. 3B). A third synthetic CB$_1$/CB$_2$ cannabinoid agonist, HU210 ($10^{-6} \, \text{M}$), did not change striatopallidal GABAergic neurotransmission (Fig. 3B).

In the next step, we wanted to determine the cannabinoid receptor subtype involved in the inhibition of neurotransmission by studying the interaction between WIN55212-2 and

![Fig. 2](https://example.com/fig2.png)

![Fig. 3](https://example.com/fig3.png)
the CB1 cannabinoid receptor antagonist rimonabant. When superfused alone for 15 min, rimonabant (10^{-6} M) did not change the amplitude of IPSCs (Fig. 4). In the presence of rimonabant, WIN55212-2 (10^{-5} M) failed to depress IPSCs (Fig. 4).

Endocannabinoid-Mediated DSI at Striatopallidal Synapses. At many synapses, depolarization of the postsynaptic neuron leads to inhibition of transmitter release from the presynaptic axon terminal. This form of retrograde signaling is termed “depolarization-induced suppression of inhibition” (DSI) in the case of GABAergic synapses and “depolarization-induced suppression of excitation” (DSE) in the case of glutamatergic synapses. DSI and depolarization-induced suppression of excitation are frequently mediated by endocannabinoids, which are synthesized and released by depolarized postsynaptic neurons (for reviews, see Wilson and Nicoll, 2002; Freund et al., 2003; Diana and Marty, 2004). We searched for DSI at striatopallidal synapses.

An intracellular solution with low-calcium buffering capacity (EGTA, 1 mM) was used in these experiments. The striatopallidal axons were stimulated in the caudate-putamen every 2 s (Fig. 5A). DSI was elicited by raising the membrane potential of globus pallidus neurons from -60 mV to -30 mV for 5 s. DSI was elicited at first in the presence of solvent, then in the presence of the antagonist rimonabant. In the

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**Fig. 4.** Interaction between WIN55212-2 (WIN) and the CB1 receptor antagonist rimonabant (RIM) on IPSCs recorded in globus pallidus neurons. IPSCs were evoked every 15 s by electrical stimulation in the caudate-putamen. IPSCs were averaged every 2.5 min (10 IPSCs) and expressed as percentages of the initial reference value (PRE). One group received SOL. The other group received RIM (10^{-6} M) plus WIN (10^{-5} M). Means ± S.E.M. of six (RIM + WIN) and 22 (SOL) experiments.

**Fig. 5.** Effect of depolarization of globus pallidus neurons on IPSCs recorded in globus pallidus neurons and intracellular calcium concentrations in globus pallidus neurons. A, IPSCs were evoked by electrical stimulation in the caudate-putamen every 2 s. IPSCs were expressed as percentages of the initial reference value (PRE); moreover, moving averages including three IPSCs were calculated. Globus pallidus neurons were depolarized from the holding potential of -60 mV to -30 mV for 5 s. The depolarization protocol was carried out in each neuron in the presence of SOL and then in the presence of rimonabant (RIM; 10^{-6} or 10^{-5} M; the effects of the two rimonabant concentrations were identical; therefore, the experiments were pooled). Means ± S.E.M. of 13 experiments. Significant difference from PRE: †, p < 0.05. Significant difference from SOL: ‡, p < 0.05. The insets show IPSCs obtained before (time point 1) and after (time point 2) depolarization in the presence of SOL and RIM (10^{-6} M). B1, after loading with the calcium-sensitive fluorescent dye Oregon Green 488 BAPTA-5N, globus pallidus neurons were depolarized from the holding potential of -60 mV to +30 mV for 5 s. Means ± S.E.M. of six experiments (for sake of clarity, only every fifth standard error bar is displayed). B2, fluorescence images of a globus pallidus neuron obtained before stimulation (time point 1) and during the maximum effect of stimulation (time point 2).
presence of solvent, the depolarization led to a small suppression of IPSCs: the maximal suppression was 27%, and the suppression was shorter than 8 s. Rimonabant was superfused at two concentrations, 10^{-6} and 10^{-5} M. Since the results obtained at the two concentrations were identical, the experiments were pooled. In the presence of rimonabant, the depolarization of the postsynaptic neuron no longer suppressed the IPSCs. Rather, a small potentiation occurred (Fig. 5A). Prevention of DSI by the cannabinoid antagonist suggests that endocannabinoids acting at CB_{1} receptors were involved.

Although it is generally accepted that endocannabinoid synthesis in postsynaptic neurons is triggered by an increase in intracellular calcium concentration, the depolarization-evoked increase in intracellular calcium concentration has been determined in only few studies (Glitsch et al., 2000; Wang and Zucker, 2001; Brenowitz and Regehr, 2003). Therefore, we decided to determine the calcium concentration increases in globus pallidus neurons. Globus pallidus neurons were loaded via the patch pipette with the low-affinity calcium indicator Oregon Green 488 BAPTA-5N (Fig. 5B). Neurons were depolarized like in experiments in which DSI was studied, i.e., from −60 mV to +30 mV for 5 s. In response to this depolarization, the calcium concentration in somatic and dendritic regions of globus pallidus neurons increased maximally to 14.5 and 9.9 μM, respectively.

Cannabinoids Inhibit Neurotransmission Presynaptically. Three kinds of experiments have been carried out to determine whether cannabinoids depressed striatopallidal neurotransmission with a pre- or postsynaptic action.

First, we tested whether WIN55212-2 interferes with the activation of postsynaptic GABA_{A} receptors on globus pallidus neurons by muscimol. Muscimol (10^{-3} M) was pressure-ejected from a pipette in the vicinity of the recorded neurons. During the initial reference period (PRE), muscimol-evoked currents had an amplitude of 268 ± 73 pA (n = 16). Figure 6 shows that the muscimol-evoked currents remained stable in solvent-treated slices. Superfusion with WIN55212-2 (10^{-5} M) did not elicit any effect (Fig. 6). Thus, WIN55212-2 did not interfere with the activation of postsynaptic GABA_{A} receptors on globus pallidus neurons.

In the second set of experiments, postsynaptic GABA_{A} receptors were activated by GABA released by photolysis of caged GABA. Slices were superfused with ACSF containing CNB-caged GABA (8 × 10^{-5} M). The recorded neuron was illuminated with flash light every 60 s. The flash elicited GABA_{A} receptor-mediated currents: the currents were abolished by bicuculline (2 × 10^{-5} M) and reversed polarity near the calculated equilibrium potential of chloride (not shown). During the initial reference period (PRE), flash-evoked currents had an amplitude of 825 ± 123 pA (n = 12). The decay time constant (τ) of flash-evoked currents was 64 ± 10 ms during the PRE period (n = 12). Flash-evoked currents remained stable in solvent-treated slices (Fig. 7, A and B). Superfusion of WIN55212-2 (10^{-5} M) affected neither the amplitude nor the time constant of flash-evoked currents (Fig. 7, A–C). Thus, WIN55212-2 did not interfere also with the activation of postsynaptic GABA_{A} receptors when these receptors were activated with fast kinetics resembling physiological conditions. At the end of the experiments, the GABA uptake inhibitor NO-711 (2 × 10^{-5} M) was superfused. It did not change the amplitude of flash-evoked currents but significantly prolonged these currents (Fig. 7, A–C). This latter observation verifies that our method is suitable to detect changes in GABA uptake.

In the third series of experiments, a traditional analysis of mIPSCs was carried out. mIPSCs were isolated by tetrodotoxin (3 × 10^{-7} M). During the initial reference period (PRE), the frequency and amplitude of mIPSCs were 2.7 ± 0.7 Hz and 68 ± 8 pA (n = 10), respectively. In control experiments with solvent, the frequency and amplitude of mIPSCs remained stable in solvent-treated slices (Fig. 7, A–C). However, NO-711 superfused. It did not change the frequency nor the amplitude of mIPSCs but significantly prolonged these currents (Fig. 7, A–C). This latter observation verifies that our method is suitable to detect changes in GABA uptake.

**Fig. 6.** Effects of WIN55212-2 (WIN) and SOL on currents evoked in globus pallidus neurons by pressure ejection of muscimol. Currents were evoked every 1 min by ejection of muscimol (10^{-3} M) from a pipette in the vicinity of the recorded neurons. Muscimol-evoked currents were averaged every 5 min (five currents) and expressed as percentages of the initial reference value (PRE). Means ± S.E.M. of eight (WIN 10^{-5} M) and eight (SOL) experiments. The inset shows muscimol-evoked currents obtained at time points 1 and 2 in a typical experiment with WIN.

**Fig. 7.** Effects of WIN55212-2 (WIN), the GABA uptake inhibitor NO-711, and SOL on currents evoked in globus pallidus neurons by GABA released by photolysis of caged GABA. The superfusion ACSF included CNB-caged GABA (8 × 10^{-5} M). The recorded neuron was illuminated with flash light every 60 s. Amplitudes and decay time constants (τ) of flash-evoked currents were expressed as percentages of the initial reference value (PRE). Means ± S.E.M. of seven (WIN, 10^{-5} M; and NO-711, 2 × 10^{-5} M) and five (SOL) experiments. Significant difference from SOL: *, p < 0.05. C, the original recordings were obtained at time points 1 to 3 (see A) in a typical experiment with WIN and NO-711. C2 and C3, PRE curves were scaled for obtaining identical amplitudes with the WIN and NO-711 curves.
mained constant (Fig. 8E). WIN55212-2 (10⁻⁵ M) changed neither the frequency (Fig. 8, A, D, and E) nor the amplitude of mIPSCs (Fig. 8, B, C, and E). Lack of effect of WIN55212-2 on the amplitude of mIPSCs indicates that the cannabinoid did not interfere with the effect of synaptically released GABA on postsynaptic globus pallidus neurons. This latter observation and the observations with muscimol and caged GABA all support—by exclusion of a postsynaptic action—a presynaptic mode of action of cannabinoids at inhibiting synaptic transmission. The lack of effect on mIPSC frequency suggests that the vesicular release machinery was not directly inhibited.

Cannabinoids Inhibit the Action-Potential Evoked Calcium Concentration Increase in Terminals of Striatopallidal Axons. It has been shown in the previous section that cannabinoids inhibit striatopallidal neurotransmission with a presynaptic action. The final aim was to characterize the mechanism of the presynaptic action in more detail. Since the vesicular release machinery was not directly inhibited, we assumed that the cannabinoids inhibited the action potential-evoked increase in calcium concentration in axon terminals. To test this hypothesis, we measured the concentration of calcium in terminals of striatopallidal axons.

Slices were prepared from brains of mice in which the striatopallidal axons had been labeled with the calcium-sensitive fluorescent dye Oregon Green 488 BAPTA-1 dextran. Striatopallidal axons were stimulated in the caudate-putamen with a short series of pulses (four pulses at 100 Hz), and the stimulation-evoked fluorescence increase was observed in the globus pallidus with an imaging camera (Fig. 9).

The stimulation elicited a weak increase in fluorescence in the globus pallidus (compare Fig. 9, B and C). The site of fluorescence increase was determined by subtraction of the image obtained before stimulation (Fig. 9B) from the image obtained after stimulation (Fig. 9C). The subtraction image shown in Fig. 9D indicates an inhomogeneous increase in fluorescence. Three ROIs were selected, and further evaluations were based on these ROIs. Figure 9E shows the time pattern of stimulation-evoked fluorescence change at the three ROIs indicated in Fig. 9D. During the initial reference period (PRE), the peak ΔF/F₀ value was 0.065 ± 0.006 (n = 48).

The effects of the cannabinoid agonist WIN55212-2 were compared with the effects of solvent (Fig. 10, A–C). In addition, the consequences of sodium channel blockade by tetrodotoxin and calcium channel blockade by cadmium were also studied (Fig. 10, A–C). During superfusion of solvent, the calcium transient did not change (Fig. 10, A and B). When tetrodotoxin was superfused at the end of the solvent experiments, it abolished the calcium transients (Fig. 10, A and B; see also Fig. 9E). In the other group, WIN55212-2 (10⁻⁵ M) was superfused: it decreased the amplitude of the calcium transients by 22% (Fig. 10, A and C). When cadmium (10⁻⁴ M) was superfused at the end of the experiments, it greatly decreased the amplitude of the transients (Fig. 10, A and C).

Discussion

This is the first study of the effect of cannabinoids on GABAergic neurotransmission between striatopallidal axons and globus pallidus neurons. The results show that activation of CB₁ cannabinoid receptors by exogenous agonists and by endocannabinoids released by globus pallidus neurons presynaptically inhibits striatopallidal synaptic transmission. Inhibition of the action potential-evoked calcium increase in the axon terminals is the basis of the presynaptic inhibition.

Striatopallidal neurotransmission was selectively activated by stimulation in the caudate-putamen. This approach allowed unambiguous localization of the cannabinoid effect to terminals of striatopallidal axons. The advantage of stimulation in the caudate-putamen versus stimulation in the globus pallidus for studying drug effects on the striatopallidal pathway has been recently shown by Cooper and Stanford (2001). Inhibition of neurotransmission by dopamine was seen only if stimulation occurred in the caudate-putamen. When the GABAergic input was stimulated in the vicinity of the recorded neurons in the globus pallidus, dopamine had only a minimal effect. Obviously, dopamine effects on the striatopallidal pathway were masked when intrapallidal GABAergic connections were additionally stimulated (Cooper and Stanford, 2001).

It is very likely that the receptors responsible for the inhibition of striatopallidal GABAergic neurotransmission are

![Figure 8](https://jpet.aspetjournals.org/)

**Fig. 8.** Effects of WIN55212-2 (WIN) and SOL on mIPSCs recorded in globus pallidus neurons in the presence of tetrodotoxin (3 × 10⁻⁷ M). mIPSCs were recorded during the initial reference period (PRE) and during superfusion with WIN (10⁻⁵ M) or SOL. A, original tracings from an experiment with WIN. B, averaged mIPSCs from an experiment with WIN (same experiment as in A). C and D, cumulative probability distribution plots of amplitudes and inter-event intervals of mIPSCs from an experiment with WIN (same experiment as in A). E, means ± S.E.M. of six (WIN) and five (SOL) experiments.
CB₁ receptors. The inhibition was elicited by the synthetic drugs WIN55212-2 and CP55940. The two drugs belong to greatly differing chemical classes, but both of them are agonists at CB₁ and CB₂ receptors (Howlett et al., 2002; Pertwee, 2005). High concentrations of WIN55212-2 and CP55940 were necessary for the inhibition of neurotransmission. The reason is very likely the poor penetration of these substances into the brain slice, as impressively demonstrated by Brown et al. (2004). HU210 (10⁻⁶ M) was ineffective in our study, although it produced effects in other brain slice studies at this concentration (e.g., Gerdean and Lovinger, 2001). It may be that the neurons recorded by us were located more deeply under the surface of the brain slice than in the other studies; this can hinder penetration of HU210 to the target neurons (see Brown et al., 2004). The CB₂-selective antagonist rimonabant (Howlett et al., 2002; Pertwee, 2005) abolished the inhibition of IPSCs by WIN55212-2: this observation verifies the involvement of CB₁ receptors. It has been recently observed that WIN55212-2 can elicit effects in the brain independently of CB₁ receptors, and a novel type of cannabinoid receptor was postulated (Breivogel et al., 2001; Hašos and Freund, 2002). Since CP55940 does not elicit such a non-CB₁ receptor-mediated effect (Breivogel et al., 2001), it is unlikely that non-CB₁ receptors played a role in the present study.

In three kinds of experiments (1–3), WIN55212-2 did not interfere with the activation of postsynaptic GABAₐ receptors. 1) WIN55212-2 did not change the amplitude of currents evoked by muscimol in globus palidus neurons. 2) Currents elicited by flash photolysis of caged GABA were also not interfered. Since postsynaptic effects by WIN55212-2 can be excluded, it is very likely that WIN55212-2 (and CP55940) inhibited striatopallidal neurotransmission with a presynaptic mechanism. A further argument for a presynaptic action
is the anatomical localization of the CB₁ receptor. The presynaptic striatopallidal medium spiny neurons synthesize CB₁ receptors, whereas the postsynaptic globus pallidus neurons generally do not (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Hohmann and Herkenham, 2000).

The experiments with flash photolysis of caged GABA indicate that WIN55212-2, at the concentration causing strong presynaptic inhibition (10⁻⁵ M), does not influence GABA uptake. In a previous study (Maneuf et al., 1996a), WIN55212-2 depressed GABA uptake in the globus pallidus; however, higher concentrations were necessary for this effect (5–20 × 10⁻⁵ M). Systemically administered cannabinoids counteract the inhibition of globus pallidus neurons elicited by electrical stimulation in the caudate-putamen (Miller and Walker, 1996); the inhibition of the striatopallidal synapse as shown in the present brain slice study is the probable basis of this in vivo cannabinoid effect.

It is thought that activation of CB₁ receptors leads to presynaptic inhibition by one of the following mechanisms (for review, see Szabo and Schlicker, 2005): opening of certain potassium channels, inhibition of voltage-dependent calcium channels, and direct interference with the vesicle release machinery. Lack of effect of WIN55212-2 on the frequency of action potential-independent mIPSCs indicates that the vesicle release machinery was not directly inhibited in the present study.

We used a novel technique for selective labeling of the striatopallidal axon terminals with a calcium-sensitive dye. The following measurements of calcium concentrations showed that cannabinoids depress the action potential-evoked increase in calcium concentration in striatopallidal axon terminals. This depression was very likely the reason for the decrease in GABA release. Although it is generally believed that cannabinoids can depress the action potential-evoked calcium influx into axon terminals, a cannabinoid-induced decrease in axon terminal calcium currents or concentrations has been demonstrated only in two brain regions, the cerebellar cortex (Diana et al., 2002; Brown et al., 2004; Daniel et al., 2004) and the brain stem (Kushmerick et al., 2004). Our experiments show that cannabinoids lower the calcium concentration in an additional region, the globus pallidus.

We did not attempt to clarify whether the depressed calcium response and the resulting inhibition of transmitter release are due to a primary action of cannabinoids on voltage-dependent calcium channels or potassium channels (potassium channel modulation can lead to changes in calcium channel activation). Some data suggest that cannabinoids cause presynaptic inhibition by primarily inhibiting calcium channels (Hoffman and Lupica, 2000; Liang et al., 2003; Brown et al., 2004). Other data point to potassium channels as the primary targets of cannabinoids causing presynaptic inhibition (Diana and Marty, 2003; Daniel et al., 2004).

The CB₁ receptor antagonist rimonabant, superfused alone, did not enhance the amplitude of IPSCs, indicating that under the conditions of the present study endocannabinoids did not tonically inhibit GABA release in the globus pallidus. Depolarization of postsynaptic globus pallidus neurons induced a suppression of the striatopallidal IPSCs; i.e., DSI occurred. Abolishment of this suppression by rimonabant indicates that endocannabinoids released from postsynaptic neurons and acting at presynaptic CB₁ cannabinoid receptors were involved in this phenomenon. The depolarization induced a robust increase in the intracellular calcium concentration in globus pallidus neurons—similar to increases observed previously in the hippocampus and the cerebellum (Wang and Zucker, 2001; Brenowitz and Regehr, 2003). Therefore, it is likely that the endocannabinoid synthesis in globus pallidus neurons was triggered by the increase in intracellular calcium concentration.

Compared with other brain regions, the extent and duration of DSI at the striatopallidal synapse was rather moderate, although the experimental conditions (age of animals, temperature during recording, composition of the intracellular solution, and duration and amplitude of the depolarizing pulse) were similar to those used in other brain regions (e.g., Wallmichrath and Szabo, 2002; Brenowitz and Regehr, 2003; Diana and Marty, 2003; Szabo et al., 2004; for reviews, see Wilson and Nicoll, 2002; Freund et al., 2003; Diana and Marty, 2004). The calcium measurements showed that the calcium concentration increased sufficiently in globus pallidus neurons. The reason for the weak DSI may be that the endocannabinoid synthesizing capacity of globus pallidus neurons is weak or that endocannabinoids do not properly diffuse to the CB₁ receptor-bearing presynaptic axon terminals. It is noteworthy that in some regions DSI even does not occur, although presynaptic CB₁ receptors are present (certain hippocampal synapses, Hoffman et al., 2003; synapses between caudate-putamen neurons, I. Freiman and B. Szabo, unpublished observations).

Cannabinoids microinjected into the globus pallidus or systemically administered cause catalepsy (Pertwee and Wikens, 1991; for review, see Sanudo-Pena et al., 1999). It has been suggested that inhibition of GABA uptake and the following enhancement of GABAergic neurotransmission in the globus pallidus is the reason for the catalepsy (Maneuf et al., 1996a,b). The present results unequivocally show that the principal effect of cannabinoids on GABAergic neurotransmission in the globus pallidus is inhibition of neurotransmission—GABA uptake was not significantly changed. Remarkably, cannabinoids also inhibit the glutamatergic subthalamopallidal neurotransmission (Freiman and Szabo, 2005). Altogether, there is no unambiguous explanation for the catalepsy induced by intrapallidal cannabinoid application. The explanation for the catalepsy elicited by systemically administered cannabinoids is even more difficult, because cannabinoids modulate GABAergic and glutamatergic neurotransmission in the basal ganglia at least at 11 sites (see Fig. 6 in Szabo and Schlicker, 2005).

In conclusion, the concentration of CB₁ cannabinoid receptors in the globus pallidus is very high. The present study unequivocally clarified the function of these receptors. Activation of CB₁ receptors on terminals of striatopallidal axons by exogenous cannabinoid agonists leads to presynaptic inhibition of GABAergic neurotransmission between these axons and globus pallidus neurons. Inhibition of the action potential-evoked increase in axon terminal calcium concentration is the event behind the presynaptic inhibition of GABA release. The presynaptic CB₁ cannabinoid receptors can also be activated by endocannabinoids released by depolarized postsynaptic globus pallidus neurons.

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References


