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Local effects of cannabinoids on spontaneous activity and evoked inhibition in the globus pallidus

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Abstract

The globus pallidus has been identified as a site of action for the motor effects of cannabinoids. A previous report from this laboratory demonstrated that systemic administration of the potent and selective cannabinoid receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl) methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl] (1-napthalenyl) methanone (WIN 55,212-2) inhibits rat pallidal neurons and reverses the inhibition of pallidal activity produced by electrical stimulation of the striatum. The current study used in vivo single unit electrophysiology/micropressure ejection to investigate whether the effects of cannabinoids on spontaneous activity and evoked inhibition in the globus pallidus are locally mediated. Micropressure ejection of either WIN 55,212-2 or CP 55,940 into the globus pallidus inhibited spontaneous activity in the globus pallidus. Local administration of the cannabinoid receptor antagonist, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A), did not produce an effect on its own but blocked the effect of WIN 55,212-2 on spontaneous activity of pallidal neurons. The decrease in pallidal activity produced by WIN 55,212-2 was not blocked by coadministration of bicuculline, suggesting this effect is independent of $GABA_A$ receptors. Micropressure ejection of cannabinoids into the globus pallidus did not reverse the inhibitory effect of striatal stimulation in the globus pallidus. Taken together, these findings suggest that pallidal cannabinoid receptors mediate an inhibition of spontaneous activity in the globus pallidus.

Keywords: Micropressure ejection; Spontaneous activity; Evoked inhibition

1. Introduction

Research over the past decade has established the existence of a cannabinoid neurotransmitter system. Howlett et al. (1988) discovered a G-protein-coupled cannabinoid receptor which was soon cloned in both rat (Matsuda et al., 1990) and man (Gérard et al., 1991). Two subtypes of cannabinoid receptor have been described: CB_1, which is found primarily within the central nervous system (Matsuda et al., 1990), and a peripheral cannabinoid receptor, CB_2 (Munro et al., 1993). The establishment of a cannabinoid neurotransmitter system was strengthened by the discovery of anandamide, an endogenous ligand for the cannabinoid receptor (Devane et al., 1992).

Binding studies with the potent and selective cannabinoid receptor agonist CP 55,940 revealed that cannabinoid receptors are densely distributed in the cerebellum and the basal ganglia (Herkenham et al., 1990; Herkenham et al., 1991a), regions associated with the regulation of motor behavior. Within the basal ganglia, cannabinoid receptors are most densely distributed in the output nuclei and in the globus pallidus. Cannabinoid receptors in the substantia nigra pars reticulata, the entopeduncular nucleus and the globus pallidus appear to be localized primarily on striatal terminals since cannabinoid receptor binding in these regions is greatly reduced following striatal lesions (Herkenham et al., 1991b).

As can be expected from the distribution of their receptors, cannabinoids profoundly affect motor behavior; in rodents, cannabinoids inhibit motor activity and produce catalepsy (Grunfeld and Edery, 1969; Holtzman et al., 1969). One region of the basal ganglia where cannabinoids act to regulate motor activity is the globus pallidus. Unilateral microinjections of cannabinoids into the globus pallidus induce ipsilateral turning (Sañudo-Peña and Walker, 1998), suggesting that cannabinoid receptors in the globus pallidus mediate an inhibition of motor activity.

Previous research in this laboratory demonstrated that intravenous administration of the cannabinoid receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpho-

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linyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl) methanone (WIN 55,212-2) inhibits the spontaneous firing of pallidal neurons (Miller and Walker, 1996). Considering the localization of pallidal cannabinoid receptors to striatopallidal terminals, it was also of interest to examine whether a cannabinoid would modulate the inhibitory effects of striatal stimulation in the globus pallidus. WIN 55,212-2 reversed the inhibition of pallidal neurons produced by striatal stimulation. These findings suggested that cannabinoids regulate both spontaneous activity and evoked inhibition in the globus pallidus, but since the cannabinoid was administered systemically, the site of action of these effects could not be determined. In the current study, local administration of cannabinoids via micropressure ejection was used to determine whether the effects of WIN 55,212-2 on spontaneous and evoked pallidal activity were mediated by cannabinoid receptors in the globus pallidus.

Recent data suggest that cannabinoids might affect pallidal activity by altering GABAergic transmission. Cannabinoids act in the globus pallidus to potentiate the cataleptic effects of benzodiazepines and muscimol (Pertwee and Wickens, 1991; Wickens and Pertwee, 1993). In addition, cannabinoids inhibit pallidal γ-aminobutyric acid (GABA) reuptake in a slice preparation in a receptor-mediated manner (Maneuf et al., 1996). Therefore, it was also of interest to determine whether the inhibition of neural activity in the globus pallidus produced by local administration of a cannabinoid receptor agonist occurred in the presence of the $GABA_A$ receptor antagonist bicuculline.

It is important to determine whether an endogenous cannabinoid tonically regulates motor activity. The observation that systemic administration of the cannabinoid receptor antagonist, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-pyrazole-3-carbamide hydrochloride (SR141716A), stimulates motor behavior (Compton et al., 1996) supports this hypothesis. Anandamide, an endogenous ligand for the cannabinoid receptor, has been detected in the striatum of humans and rats (Felder et al., 1996), but a potential role of endogenous cannabinoids in the globus pallidus remains previously unexplored. Therefore, this study also examined whether local administration of SR141716A affects spontaneous activity in the globus pallidus.

2. Materials and methods

2.1. Drugs and chemicals

WIN 55,212-2 was purchased from Research Biochemicals International (Natick, MA); WIN 55,212-3 was a gift from Sterling-Winthrop. WIN 55,212-3 is the inactive enantiomer of WIN 55,212-2 (D'Ambra et al., 1992) and was used to control for non-receptor-mediated effects of WIN 55,212-2. SR141716A was generously provided by Sanofi (Montpellier, France) and NIDA (Rockville, MD). CP 55,940 was a gift from Pfizer (Groton, CT). Bicuculline methiodide was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in saline. WIN 55,212-2, WIN 55,212-3, CP 55,940 and SR141716A were dissolved in 45% 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International) at a concentration of 2 mg/ml (3.8 mM for WIN 55,212-2 and WIN 55,212-3, 5.3 mM for CP 55,940 and 3.8 mM for SR141716A). Drug vials were siliconized with Sigmacote (Sigma-Aldrich) in order to prevent the drugs from adhering to the surfaces.

2.2. Animals and surgical preparation

Male Sprague–Dawley albino rats ($n = 58$, 250–350 g) were anesthetized with a 25% solution of urethane in normal saline (1.25 g/kg, i.p.). The rat's body temperature was maintained at 37°C throughout the experiment. A craniotomy was performed at coordinates of 0.8–1.2 mm posterior to bregma and 2.5–3.5 mm lateral to the midline (derived from Paxinos and Watson, 1986).

2.3. Electrophysiological recordings

Three-barrel electrode blanks were constructed from 1.5 mm omega dotstock glass filaments (Glass Co. of America, Millville, NJ) fastened at each end with metal tubing and epoxy. Electrodes were prepared using a Narishige PE2 puller, and the tips were broken back under a microscope to 3–4 μm. One barrel of the electrode assembly was filled with fast green dye for recording. For each cell, the two micropressure ejection barrels respectively contained either: WIN 55,212-2 and vehicle, WIN 55,212-2 and WIN 55,212-3, WIN 55,212-2 and SR141716A or CP 55,940 and vehicle. In experiments using bicuculline, one barrel was filled with a 10-mM solution of bicuculline methiodide for iontophoresis. The electrode was lowered into the brain using a Kopf hydraulic microdrive. Amplified action potentials were passed through low and high pass filters into a window comparator that produced a logic pulse for each action potential which was passed to a computer. Electrical signals were monitored on an audio amplifier and displayed on an oscilloscope.

Following each experiment, fast green dye was ejected from the recording electrode tip as an anion by iontophoresis (5–30 mA, 20 min). Animals were sacrificed and perfused with 10% formalin. Brains were frozen, sectioned at 40 μm and stained with neutral red, and all recording sites were verified microscopically.

2.4. Pressure ejection of cannabinoids on spontaneous activity in the globus pallidus

The first set of experiments examined whether micropressure ejection of cannabinoids into the globus pallidus would affect spontaneous neural firing in the globus pal-

lidus. For each cell, the two micropressure ejection barrels contained either: WIN 55,212-2 and vehicle, WIN 55,212-2 and WIN 55,212-3, or CP 55,940 and vehicle. Order of drug/vehicle administration was counterbalanced. Following a 3–5-min baseline, the drug or vehicle was pressure-ejected for 3 min at 0.2–3.6 kg/cm^2. The optimal pressure for ejection was determined for each barrel by observing the lowest pressure necessary to disrupt the recording of a sample cell; a slightly lower pressure was then utilized in the experiment.

The cannabinoid receptor antagonist, SR141716A, was pre- and coadministered with WIN 55,212-2 in order to determine whether an effect observed with WIN 55,212-2 was mediated by pallidal cannabinoid receptors. SR141716A was administered first for 3 min then coadministered with WIN 55,212-2 for 3 min. After a 10-min washout period, WIN 55,212-2 was administered for 3 min.

2.5. Pressure ejection of WIN 55,212-2 in the presence of bicuculline

In order to determine whether the effect of locally-administered WIN 55,212-2 on spontaneous activity in the globus pallidus is mediated by pallidal $GABA_A$ receptors, WIN 55,212-2 was pressure-ejected in the presence of iontophoretically-administered bicuculline, a $GABA_A$ receptor antagonist. Bicuculline methiodide (10 mM) was administered by iontophoresis. This concentration was used because it was difficult to obtain a stable increase in pallidal firing with bicuculline without compromising the quality of the recording at higher concentrations of bicuculline. Iontophoretic currents ranged from 15–25 nA; a 10–20-nA retention current was applied between periods of iontophoresis.

For each cell, WIN 55,212-2 was administered both separately and together with bicuculline. The order of administration of these two drug combinations (i.e., WIN 55,212-2 in the presence and absence of bicuculline) was counterbalanced, and periods of administration were separated by 10-min intervals. In order to examine the effect of WIN 55,212-2 on spontaneous firing in the globus pallidus in the presence of bicuculline, bicuculline was first administered until the increase in firing had stabilized for at least 1 min. Iontophoresis of bicuculline continued while WIN 55,212-2 was pressure-ejected over a 3-min period.

2.6. Pressure ejection of cannabinoids on striatal stimulation-evoked inhibition in the globus pallidus

Additional experiments were carried out to determine whether pressure ejection of WIN 55,212-2 or CP 55,940 would modify the inhibitory effect of striatal stimulation on neural firing in the globus pallidus. The striatum was electrically stimulated while activity was recorded in the globus pallidus using methods based on those described by Waszczak (1990). Stimulating electrodes were constructed from stainless steel insect pins (No. 00; 0.25 mm diameter) insulated with Epoxylite, except for 0.5 mm exposed at the tip. A bipolar electrode (tips separated by 0.2 mm) was lowered 1.5 mm into the striatum (1.0 mm anterior to bregma and 1.8 and 3.8 mm lateral to the midline). Trains (500 ms) of 300–500 μA square pulses (300 μs duration, 45 Hz) were delivered to the striatum at 20-s intervals using a Grass (Quincy, MA) model S-88 stimulator and constant current photoisolation unit.

A computer recorded the time of occurrence (to 0.1 ms accuracy) of each action potential for a prestimulation period of 1 s, sent a signal to the stimulator which produced the train of pulses, and continued to record the time of occurrence of each action potential for a post-stimulation period of 2.5 s. Striatal stimulation produced a brief inhibition of neural activity in the globus pallidus. Striatal stimulation failed to alter the firing rate of some neurons, presumably due to the location of the stimulating electrodes. In such cases, a different neuron was selected. Following a baseline period during which 10 stimulations were delivered, either WIN 55,212-2, CP 55,940 or vehicle was locally applied via micropressure ejection for 3 min. Each cell received either WIN 55,212-2 or CP 55,940 and vehicle, and the order of drug/vehicle administrations was counterbalanced between cells. At the end of each experiment, a direct current (300–500 μA) was passed through the stimulating electrodes for 20 s to mark their locations within the brain. Recording and histological techniques were performed as described above.

2.7. Data analyses

Experiments that examined spontaneous firing were analyzed by calculating the mean firing rate during the baseline period and during each of the 3 min of pressure ejection. Rates were expressed as the percent changes from baseline firing rate and were subjected to a repeated measures analysis of variance (ANOVA; BMDP Statistical Software, Los Angeles, CA). Separate analyses were performed for each of the following groups: WIN 55,212-2 vs. vehicle, WIN 55,212-2 vs. WIN 55,212-3, and CP 55,940 vs. vehicle. A separate analysis of variance also examined whether SR141716A affected spontaneous pallidal activity and blocked the effect of WIN 55,212-2 on neural activity in the globus pallidus.

Because of the effect of bicuculline on baseline firing rates, analysis of covariance (ANCOVA) was used to determine whether iontophoretically applied bicuculline attenuated the effect of locally administered WIN 55,212-2 on spontaneous activity in the globus pallidus. This analysis compared the mean baseline firing rate and the mean firing rates during each minute of drug administration. The covariate in the analysis was the mean firing rate during the minute prior to the administration of the cannabinoid agonist.

Striatal stimulation experiments were analyzed by calculating the mean firing rate during the 100-ms period following the termination of striatal stimulation for the baseline period and during each minute of pressure ejection. Mean prestimulation firing rates were also determined for each minute of drug or vehicle administration. Values were expressed as percent of pre-stimulation firing rate during each minute. Separate ANOVAs were used to examine the effects of WIN 55,212-2 and CP 55,940 on evoked inhibition in the globus pallidus. Drug effects were analyzed by two-way repeated measures ANOVA. Differences between individual means were analyzed using *t*-tests with the Bonferroni adjustment to maintain experiment-wise error rate of $P < 0.05$.

3. Results

3.1. Local effect of WIN 55,212-2 on spontaneous firing in the globus pallidus

Micropressure ejection of WIN 55,212-2 inhibited firing in the globus pallidus over a 3-min period as compared to either vehicle: $n = 6$; $F(1,6) = 7.70$, $P < 0.05$ or the inactive enantiomer, WIN 55,212-3: $n = 7$; $F(1,5) = 12.94$, $P < 0.05$ (Figs. 1 and 2). On average, the greatest effect of WIN 55,212-2 as compared to either vehicle or WIN 55,212-3 occurred during the third minute of pressure ejection (average decrease ± S.E.M. = 18 ± 4%).

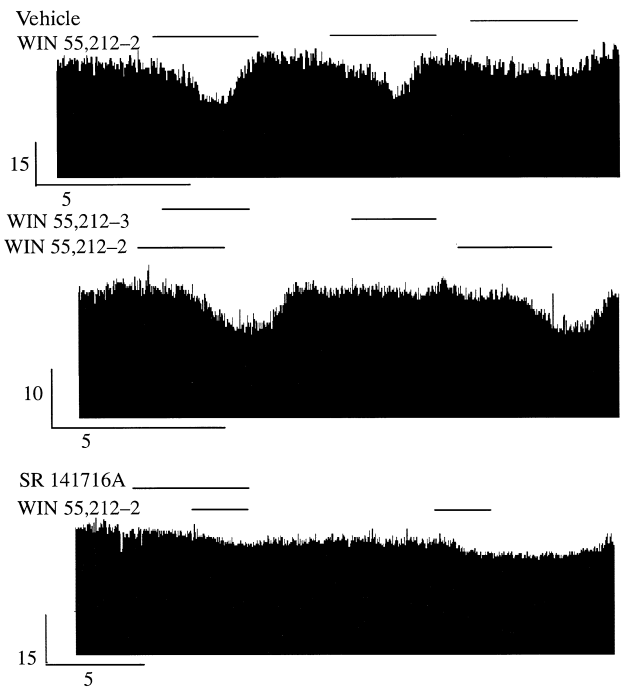

Fig. 1. Inhibition of firing in the globus pallidus produced by WIN 55,212-2 (all panels) but not by the vehicle (top panel) and or the inactive inantiomer WIN 55,212-3 (middle panel). The effect of WIN 55,212-2 was blocked by pre- and coadministration of the cannabinoid receptor antagonist SR141716A (bottom panel).

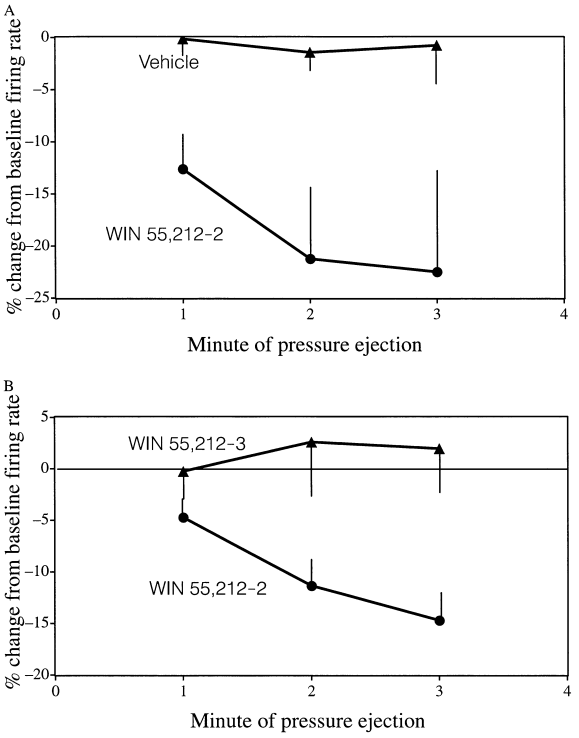

Fig. 2. Effect of micropressure ejection of WIN 55,212-2 on spontaneous activity in the globus pallidus. WIN 55,212-2 inhibited spontaneous activity in the globus pallidus during the first 3 min of micropressure ejection ($P < 0.05$ panels A and B). Neither the vehicle (panel A) nor the inactive enantiomer (panel B) produced this effect.

When administered alone, the cannabinoid receptor antagonist SR141716A did not alter the firing rate of pallidal neurons during 3 min of micropressure ejection. However, SR141716A blocked the effect of WIN 55,212-2 on spontaneous activity when coadministered for an additional 3 min, since the decrease in firing rate produced by WIN 55,212-2 in the absence of SR141716A was significantly greater than that produced in its presence: $n = 7$; $F(1,6) = 12.35$, $P < 0.025$ (Figs. 1 and 3).

3.2. Local effect of CP 55,940 on neural firing in the globus pallidus

Micropressure ejection of CP 55,940 inhibited firing in the globus pallidus over a 3-min period as compared to vehicle: $n = 9$; $F(1,8) = 7.16$, $P < 0.05$ (Fig. 4). On average, the greatest effect of CP 55,940 occurred during the third minute of pressure ejection (average decrease ± S.E.M. = 18 ± 3%).

3.3. Lack of effect of bicuculline on inhibition of spontaneous firing in the globus pallidus produced by WIN 55,212-2

ANCOVA revealed that, although bicuculline increased the firing rate of neurons in the globus pallidus by an

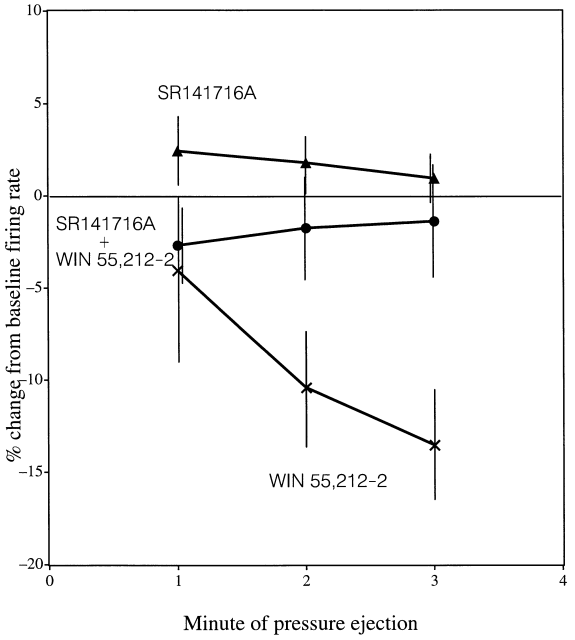

Fig. 3. Blockade of cannabinoid agonist-induced inhibition of spontaneous activity in the globus pallidus by the cannabinoid antagonist SR141716A. The cannabinoid receptor antagonist SR141716A failed to significantly affect firing on its own during 3 min of micropressure ejection, but it blocked the effect of WIN 55,212-2 on spontaneous activity when coadministered for an additional 3 min ($n = 7$; $P < 0.025$). After a 10-min washout period, WIN 55,212-2 inhibited spontaneous activity in the globus pallidus.

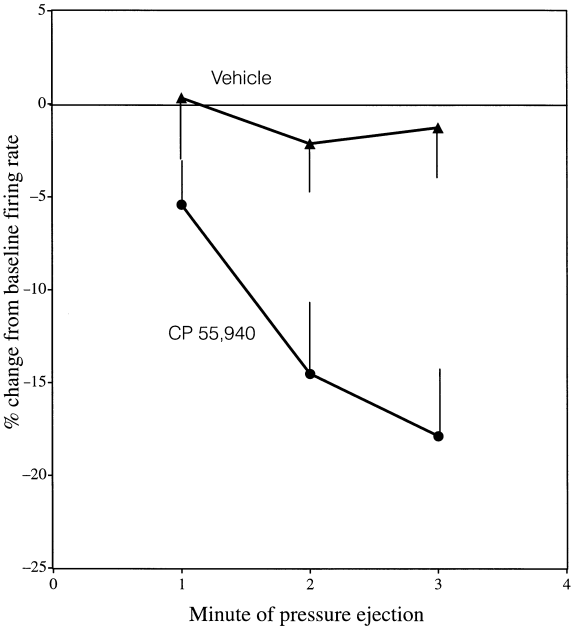

Fig. 4. Effect of micropressure ejection of CP 55,940 on spontaneous activity in the globus pallidus. CP 55,940 inhibited spontaneous activity in the globus pallidus during the first 3 min of micropressure ejection ($n = 9$; $P < 0.05$).

average of $66 \pm 6\%$, the effect of WIN 55,212-2 did not differ in the presence and absence of bicuculline: $n = 17$; $F(1,13) = 1.07$, $P > 0.05$.

3.4. Lack of effect of WIN 55,212-2 and CP 55,940 on evoked firing

Striatal stimulation produced a mean $55 \pm 5\%$ decrease in pallidal firing in the first 100 ms following stimulation. Neither WIN 55,212-2 ($n = 6$) nor CP 55,940 ($n = 6$), produced a significant effect on evoked inhibition in the globus pallidus ($P > 0.05$).

4. Discussion

Previous work in this laboratory demonstrated that intravenous administration of the cannabinoid receptor agonist, WIN 55,212-2, inhibits spontaneous activity in the globus pallidus and reverses the inhibition of neural activity produced by electrical stimulation of the striatum (Miller and Walker, 1996). The current study extended these findings by examining whether these effects on spontaneous and evoked pallidal activity are mediated by cannabinoid receptors in the globus pallidus. In addition, the current study included use of the potent and selective cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR141716A. Local administration of two cannabinoid receptor agonists, WIN 55,212-2 and CP 55,940, caused inhibition of spontaneous activity in the globus pallidus. The failure of WIN 55,212-3, the inactive enantiomer of WIN 55,212-2, to mimic this effect and the failure of WIN 55,212-2 to produce the effect in the presence of SR141716A suggest that the inhibitory effect of intrapallidal cannabinoids on spontaneous activity was mediated by cannabinoid receptors. Furthermore, the effect of intrapallidal cannabinoids on spontaneous firing suggests that cannabinoid receptors in the globus pallidus mediate the inhibition of pallidal firing observed following systemic administration of a cannabinoid but does not rule out a contribution from other brain areas.

In some cells, the firing rate recovered during the third minute of pressure ejection (e.g., see Fig. 1, top panel). Rapid desensitization to the effect of local administration of cannabinoids on cell firing has also been observed in the substantia nigra pars reticulata (Tersigni and Rosenberg, 1996). This desensitization might be due to the internalization of cannabinoid receptors which has been reported to occur in AtT20 cells expressing rat cannabinoid CB_1 receptors (Mackie et al., 1997).

If cannabinoids inhibit GABA reuptake in the globus pallidus, as has been demonstrated in vitro by Maneuf et al. (1996), then one would expect local administration of cannabinoids into the globus pallidus to inhibit pallidal activity, since inhibition of GABA reuptake would en-

hance the inhibitory effect of GABA in the globus pallidus. Indeed, the current study revealed an inhibition of spontaneous pallidal activity following micropressure ejection of cannabinoids into the globus pallidus. However, the failure of bicuculline coadministration to attenuate the effect of WIN 55,212-2 on spontaneous pallidal activity does not support the hypothesis that cannabinoids affect pallidal activity by altering GABAergic transmission. Perhaps a more complete blockade of pallidal $GABA_A$ receptors would attenuate the effect of WIN 55,212-2, but this was not possible because iontophoresis of higher concentrations of bicuculline produced unstable increases in firing rate and decreases in the size of the action potentials which made recording difficult. An alternative mechanism through which pallidal cannabinoids may act to inhibit spontaneous activity in the globus pallidus is by inhibiting release of excitatory amino acids from the subthalamic nucleus into the globus pallidus in a manner similar to that which apparently occurs in the substantia nigra pars reticulata (Sañudo-Peña and Walker, 1997; Sañudo-Peña et al., 1996). Cannabinoids also have been shown to inhibit the release of glutamate in hippocampal cultures (Shen et al., 1996).

In contrast to the results obtained on spontaneous activity, intrapallidal administration of WIN 55,212-2 and CP 55,940 failed to mimic the effect of an intravenously administered cannabinoid on striatal stimulation-evoked inhibition of pallidal neurons. Intrapallidal administration of cannabinoids also failed to increase the duration of inhibition (data not shown), which would have been expected if cannabinoids inhibit GABA reuptake in the globus pallidus. Therefore, these data do not support the hypothesis the cannabinoid-mediated reversal of inhibition produced by striatal stimulation observed previously (Miller and Walker, 1996) is mediated by receptors in the pallidum. However, an effect on the afferent terminals cannot be ruled out either, since it may require greater local concentrations of cannabinoids than were achieved in this experiment, especially if cannabinoids produce an effect at a presynaptic site distant from the soma. In this case, the local concentration of cannabinoids required to produce an effect on evoked inhibition would be higher than that required to alter spontaneous activity. Alternatively, the effect of intravenous administration of cannabinoids on evoked activity may be due to effects of cannabinoids in other brain regions, such as the striatum, where cannabinoids might act on K^+ channels to hyperpolarize striatal neurons as described for hippocampal neurons by Deadwyler et al. (1993) and make them less excitable.

Taken together, the findings from the current study suggest that cannabinoid receptors in the globus pallidus depress the spontaneous activity of pallidal neurons. The ability of intrapallidal cannabinoids to inhibit neural activity in the globus pallidus provides a cellular basis for the ipsilateral turning behavior induced by unilateral microinjection of a cannabinoid receptor agonist into the globus pallidus (Sañudo-Peña and Walker, 1998).

The failure of intrapallidal administration of SR141716A to affect cellular activity in the globus pallidus suggests that endogenous cannabinoids may not modulate the spontaneous firing of pallidal neurons. However, behavioral observations following microinjection of SR141716A into the globus pallidus should be carried out to further examine this hypothesis, since an interaction with anesthesia cannot be ruled out. The finding that micropressure ejection of cannabinoids into the globus pallidus inhibits pallidal activity through interaction with cannabinoid receptors suggests that pallidal cannabinoid receptors might inhibit motor behavior under circumstances in which endogenous cannabinoids are released into the globus pallidus.

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