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Effects of a cannabinoid on spontaneous and evoked neuronal activity in the substantia nigra pars reticulata

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Abstract

Single unit electrophysiology was used to explore the role of cannabinoid receptors in the substantia nigra pars reticulata. Intravenous and intraperitoneal injections of the potent and selective synthetic cannabinoid (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl) methanone (WIN 55,212-2) produced modest but significant increases in the spontaneous firing rate of neurons in the substantia nigra pars reticulata. In a second set of experiments, WIN 55,212-2 (up to 1.0 mg/kg i.v.) antagonized the inhibition of firing produced in the substantia nigra pars reticulata by electrical stimulation of the striatum. The pharmacological specificity of this effect was demonstrated using the inactive enantiomer WIN 55,212-3. The possibility that WIN 55,212-2 exerts its effects by regulating γ-aminobutyric acid (GABA) release from striatonigral fibers was suggested by the observation that bicuculline (up to 0.5 mg/kg i.v.) reversed the effect of striatal stimulation. It thus appears that cannabinoid receptors on striatonigral neuron terminals may regulate movement by disinhibiting the activity of substantia nigra pars reticulata neurons, perhaps by inhibiting the release of GABA into the substantia nigra pars reticulata.

Keywords: Basal ganglia; Cannabinoid; Substantia nigra; Cannabinoid receptor; Motor system; Marijuana; Tetrahydrocannabinol; WIN 55,212-2; Aminoalkylindole

1. Introduction

The significance of cannabinoid pharmacology for understanding brain function was firmly established by the discovery of a G-protein coupled cannabinoid receptor (Devane et al., 1988) and the identification of putative endogenous cannabinoids (Devane et al., 1992; Evans et al., 1992,1994). Although the literature on cannabinoid pharmacology (reviewed by Dewey, 1986; Martin, 1986) provides some clues about the possible functions of this novel endogenous system, little is known at present. Nevertheless, several lines of evidence suggest the possible role of endogenous cannabinoids in the regulation of movement. In this regard, it is noteworthy that the potent and selective cannabinoid receptor agonist [^{3}H]CP55,940 binds to a dense population of cannabinoid receptors in rat basal ganglia. Cannabinoid receptors are most concentrated in the outflow nuclei: the internal segment of the globus pallidus and the substantia nigra pars reticulata (Herkenham et al., 1991a,b; Jansen et al., 1992). The density of cannabinoid receptors in these areas is comparable to the density of dopamine receptors in striatum or glutamate receptors in cortex (Herkenham et al., 1991a,b), an observation that suggests the potential importance of cannabinoid receptors in the regulation of movement.

As one would predict from the high density of cannabinoid receptors within the basal ganglia, cannabinoids produce profound motor effects (Abood and Martin, 1992). Low doses of cannabinoids produce both stimulation and depression of movement (Garriott et al., 1967). Increasing the dose causes decreased motor activity, which is often accompanied by hyperreflexia; increasing the dose further results in catalepsy (Grunfeld and Edery, 1969; Holtzman et al., 1969).

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Because systemic administration of cannabinioids produces profound motor effects through an interaction with cannabinoid receptors (Compton et al., 1993), it appears likely that endogenous cannabinoids serve normally to modulate motor activity as well.

Microinjections of cannabinoids revealed that the basal ganglia mediate at least some of the motor effects of cannabinoids. Bilateral microinjection of cannabinoids into the striatum produces catalepsy (Gough and Olley, 1978). Likewise, microinjection of Δ^9-tetrahydrocannabinol into the posterior medial region of the globus pallidus results in mild but significant catalepsy, and this effect is potentiated by the benzodiazepine chlordiazepoxide (Pertwee and Wickens, 1991). These findings suggest that endogenous cannabinoids may exert a powerful influence on motor signals generated within the basal ganglia.

Within the basal ganglia, cannabinoid receptors occur both pre- and postsynaptically. Studies using in situ hybridization (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993) confirmed the results from binding studies showing the presence of mRNA for cannabinoid receptors in the striatum. The presence of cannabinoid receptors on striatonigral terminals was suggested by two findings: (1) [^{3}H]CP55,940 labels cannabinoid receptors in the substantia nigra pars reticulata, but cannabinoid receptor mRNA appears to be absent from this structure (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993); and (2) [^{3}H]CP55,940 binding in the substantia nigra pars reticulata disappears following lesions of the caudate nucleus (Herkenham et al., 1991b). It thus appears that cannabinoid receptors are located primarily on the terminals of striatonigral projections (Herkenham et al., 1991b), a conclusion that is consistent with the loss of cannabinoid receptors in the substantia nigra pars reticulata in Huntington's disease (Glass et al., 1993).

Nothing is known about the physiological actions of cannabinoids within the substantia nigra. One mechanism through which cannabinoids might affect receptors on the striatonigral terminals is through regulation of the cAMP second messenger system. Cannabinoids have been shown to inhibit adenylate cyclase through a G-protein coupled receptor (Howlett et al., 1987; Bidaut-Russell et al., 1990). Another mechanism through which cannabinoids might affect the presynaptically located receptors in the substantia nigra pars reticulata was suggested by Mackie and Hille (1992) who reported that cannabinoids inhibit N-type Ca^{2+} channels in NG108-15 cells. Because of the documented role of N-type Ca^{2+} channels in neurotransmitter release (Dooley et al., 1987; Miller, 1987), cannabinoids would be expected to decrease the release of neurotransmitters from striatonigral neurons. If this occurred, one would expect to observe a disinhibition of substantia nigra pars reticulata neurons, because of the predominantly inhibitory influence of γ-aminobutyric acid (GABA) and prodynorphin products, which are released from striatonigral neurons (Precht and Yoshida, 1971; Crossman et al., 1973; Khachaturian et al., 1982; Vincent et al., 1982a,b; Fallon et al., 1985). Therefore, one would predict (1) that administration of cannabinoids would increase the spontaneous firing of substantia nigra pars reticulata neurons and (2) that administration of cannabinoids would decrease GABA-mediated inhibition of neural firing in the substantia nigra pars reticulata that occurs when the striatum is electrically stimulated. The present experiments examined these possibilities with (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]-pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl) methanone (WIN 55,212-2, D'Ambra et al., 1992) and established the pharmacological specificity with its inactive enantiomer WIN 55,212-3.

2. Materials and methods

2.1. Drugs and chemicals

WIN 55,212-2 was purchased from Research Biochemicals (Natick, MA, USA); WIN 55,212-3 was a gift from Sterling-Winthrop. Bicuculline was purchased from Sigma-Aldrich (St. Louis, MO, USA). For experiments that examined the effects of WIN 55,212-2 on spontaneous firing of substantia nigra pars reticulata neurons, the drug was prepared in an ethanol/methanesulfonic acid/saline solution (0.21% : 0.04% : 99.75%). For all other experiments, WIN 55,212-2 and WIN 55,212-3 were suspended in an ethanol/alkamuls (emulphor)/saline solution (1 : 1 : 18). Test tubes and syringes were siliconized with Sigmacote (Sigma-Aldrich, St. Louis, MO, USA) in order to prevent the drugs from adhering to the surfaces. Bicuculline (base) was dissolved in HCl and saline, and the pH was elevated to 4 with NaOH.

2.2. Animals and surgical preparation

Male Sprague-Dawley albino rats ($n = 49$, 250–350 g) were anesthetized with an 8% solution of chloral hydrate in normal saline (400 mg/kg i.p.) and supplemented as necessary through the lateral tail vein. The rat's body temperature was maintained at 37° C throughout the experiment. A craniotomy was performed above the right midbrain at coordinates of 2.7–3.2 mm anterior to lambda and 2.0–3.0 mm lateral to the midline.

2.3. Electrophysiological recordings

Single barrel glass electrodes were prepared from 2.0 mm omega dotstock (Glass Co. of America, Mill-

ville, NJ, USA) using a Narashige PE2 puller, and the tips were broken back under a microscope to 1–2 μm. The electrode was lowered into the midbrain using a Kopf hydraulic microdrive. Amplified action potentials were passed through low and high pass filters into a window comparator and a computer. Electrical signals were monitored on an audio amplifier and displayed on an oscilloscope.

A substantia nigra pars reticulata neuron was identified by its depth from the brain surface (7.5–8.0 mm), a firing rate of 8–40 Hz, an action potential of less than 1 ms in duration, and its position ventral to the dopamine neurons of the substantia nigra pars compacta, which display much longer action potentials and low frequency irregular spontaneous discharges (Bunney et al., 1973; Deniau et al., 1978; Grace and Bunney, 1979; Grace et al., 1980). Following each experiment, fast green dye was ejected from the recording electrode tip as an anion by iontophoresis (5–30 μA, 20 min). Animals were killed and perfused with 10% formalin. Brains were frozen, 40 μm frozen sections were obtained and stained with neutral red, and all recording sites were verified microscopically.

2.4. Effect of WIN 55,212-2 on spontaneous firing rate

WIN 55,212-2 (10 mg/kg i.p.) or vehicle was injected following a 5–10 min baseline. The mean firing rates were determined for the 5th and 10th min post-injection and were expressed as percentage change from baseline firing rate. In separate experiments, WIN 55,212-2 was injected i.v. following a 5–10 min baseline. For these experiments, a dose of the drug was injected via the lateral tail vein every min to achieve cumulative doses of 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg/kg. The mean firing rates were determined for the last 2.5 min of the baseline period and for the 1 min following each injection. Repeated measures ANOVA was used to analyze the data from these experiments.

2.5. Striatal stimulation studies

A second set of experiments was carried out in order to determine whether WIN 55,212-2 would modify the inhibitory effect of striatal stimulation on neural firing in the substantia nigra pars reticulata. The striatum was electrically stimulated while activity was recorded in the substantia nigra pars reticulata using methods based on those described by Waszczak (1990). A 2 × 2 mm array of four electrodes was lowered into the striatum at a location of 2.0 and 4.0 mm lateral to the midline, 8.7 and 10.7 mm anterior to lambda, and between 5.0 and 5.5 mm ventral. Stimuli were applied to three of the electrodes and the fourth served as a return path, thus allowing stimulation of a large area within the striatum. Each electrode was constructed from formvar insulated stainless steel wire, except for 0.5 mm exposed at the tip. Trains of 300–500 μA square pulses (500 ms, 300 μs duration, 45 Hz) were delivered to the striatum at 20 s intervals using a Grass (Quincy, MA, USA) 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 substantia nigra pars reticulata. In some cells, striatal stimulation did not have an effect, presumably due to the location of the stimulating electrodes; in such cases, a different cell was selected. Following a baseline period during which 20 stimulations were delivered, either WIN 55,212-2, WIN 55,212-3, or vehicle was injected intravenously (cumulative doses of 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/kg). Five stimulation trials were carried out following each injection. 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.

A set of experiments was performed in order to determine whether striatal stimulation-evoked inhibition in the substantia nigra pars reticulata was GABA-mediated. Following a baseline period during which ten stimulations were administered as described above, either bicuculline or vehicle was injected intravenously (0.25–0.5 mg/kg, cumulative). Ten stimulation trials were performed at each dose.

2.6. Data analyses

For experiments that examined the effects of WIN 55,212-2 or vehicle on spontaneous firing of substantia nigra pars reticulata neurons, the average firing rates during the 5th and 10th min post-injection were determined for each cell. Rates were expressed as the percentage change from baseline firing rate and were subjected to a repeated measures analysis of variance (ANOVA, BMDP Statistical Software, Los Angeles, CA, USA).

For the striatal-stimulation experiments, mean firing rate was determined under baseline and drug or vehicle conditions for each cell during the 100 ms period beginning 40 ms after the termination of striatal stimulation. This method allowed us to exclude data from the brief excitatory effect of stimulation that was observed in some neurons. For the bicuculline experiments, values were expressed as percentages of pre-stimulation firing rate at each dose, because bicuculline increased pre-stimulation firing rate. Drug ef-

fects were analyzed by means of a two-way repeated measures ANOVA.

In order to eliminate the possibility of spurious significant P estimates that can occur in the repeated factor analysis of variance when the assumption of homogeneity of variance and covariance is violated, the conservative Greenhouse-Geisser correction was applied to degrees of freedom for repeated factors whenever the violation of sphericity test was significant. The Dunnet control group comparison test was used in post-hoc analyses to determine which doses were effective. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Effects of systemically administered WIN 55,212-2 on spontaneous firing of substantia nigra pars reticulata neurons

Intraperitoneal injection of 10 mg/kg WIN 55,212-2 produced a small but significant increase in the spontaneous firing rate of neurons in the substantia nigra pars reticulata: Fig. 1, $n = 13$; $F(1,11) = 4.91$; $P < 0.05$. This effect was apparent within 10 min of injection. In a separate experiment using i.v. administration, WIN 55,212-2 again produced a dose-dependent increase in the spontaneous firing rate of substantia nigra pars reticulata neurons compared to baseline firing rate: $n = 8$; $F(5,35) = 9.16$; $P < 0.01$, Fig. 1. Post-hoc analysis revealed a significant increase at 1.0 mg/kg ($P < 0.05$). At this dose i.v. WIN 55,212-2 produced a 25.6 ± 2% increase over baseline firing in the substantia nigra pars reticulata compared to a 15.5% increase following i.p. administration of 10 mg/kg.

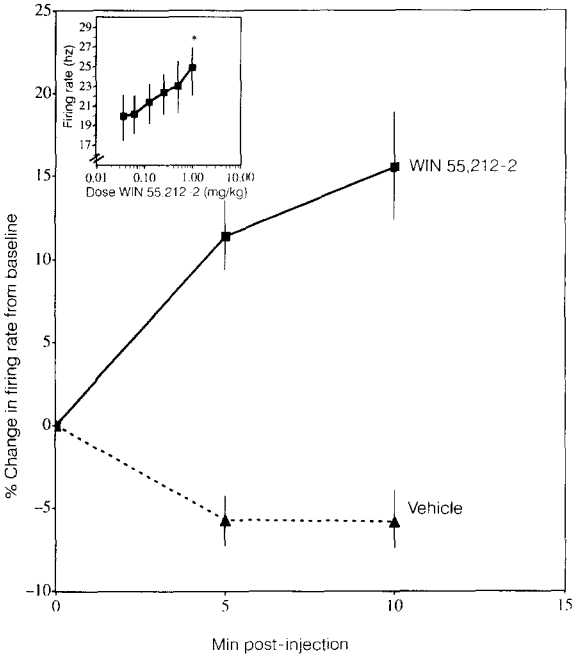

Fig. 1. Effect of WIN 55,212-2 (10 mg/kg i.p.) on the spontaneous firing rate of substantia nigra pars reticulata neurons. WIN 55,212-2 produced a modest increase in the spontaneous firing rate of substantia nigra pars reticulata neurons over the first 10 min post-injection ($n = 13$, $P < 0.05$). Inset: Effect of WIN 55,212-2 (cumulative doses of 0.0625–1.0 mg/kg) on the spontaneous firing rate of substantia nigra pars reticulata neurons. WIN 55,212-2 produced a dose-dependent increase in the spontaneous firing rate of substantia nigra pars reticulata neurons compared to baseline firing rate ($n = 8$, $P < 0.01$; * $P < 0.05$).

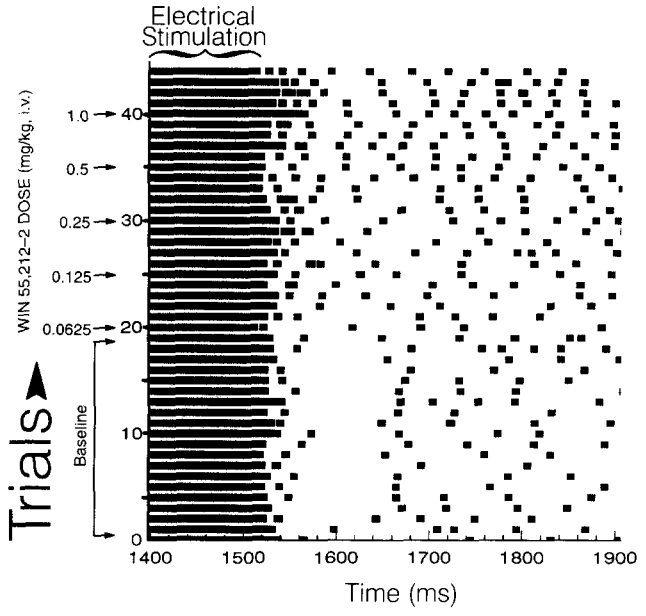

Fig. 2. Raster plot showing the effect of WIN 55,212-2 on the inhibition of a substantia nigra pars reticulata neuron evoked by electrical stimulation of the striatum. Each square represents the time of occurrence of a single action potential. Each row represents one stimulation trial. Striatal stimulation produced a brief inhibition of activity in the substantia nigra pars reticulata which was most pronounced during the first 100 ms following stimulation. This is seen as the lack of action potentials (squares) between 1540 and 1640 ms in the lower half of the record (period prior to drug administration). WIN 55,212-2 injections began following the 20th trial (cumulative doses of 0.0625–1.0 mg/kg) and were repeated every fifth trial. Note the reversal of inhibition following injection of WIN 55,212-2.

3.2. Effects of WIN 55,212-2 (i.v.) on striatal-stimulation evoked inhibition of firing of substantia nigra pars reticulata neurons

As reported by others (Precht and Yoshida, 1971; Dray et al., 1976), striatal stimulation produced a brief (approximately 100 ms) inhibition of the firing of neurons in the substantia nigra pars reticulata (Fig. 2). Under pre-drug (or pre-vehicle) conditions, striatal stimulation inhibited the firing of substantia nigra pars reticulata neurons by a mean (±S.E.M.) value of 63 ± 4% for all cells combined. The initial pre-stimulation firing rates for the three groups were not significantly

different (average ± S.E.M. = 20.93 ± 1.4), and there was no statistically significant difference between rats treated with vehicle and those treated with the inactive enantiomer WIN 55,212-3 at any dose. The cannabinoid receptor agonist WIN 55,212-2 dose-dependently attenuated the stimulation-evoked inhibition when compared to the vehicle and the inactive enantiomer: Fig. 3, $n = 24$; $F(2,56) = 3.66$; $P < 0.05$. Post-hoc analysis revealed that this effect was significant at the dose of 0.5 mg/kg (compared to vehicle) and at the doses of 0.125, 0.25, 0.5 and 1.0 mg/kg (compared to WIN 55,212-3). Reversal was complete in four out of the eight cells tested with WIN 55,212-2. Partial attenuation (29-65%) of stimulation-evoked inhibition was observed in three of the four remaining cells.

Repeated measures analysis of variance failed to reveal a significant effect of WIN 55,212-2 on pre-stimulation firing rate, as one may have expected from the previous experiments that examined spontaneous firing ($P > 0.05$).

3.3. Intravenous injection of bicuculline following striatal stimulation

Bicuculline (0.25–0.5 mg/kg i.v.) produced a dose-dependent increase in the rate of spontaneous discharge of substantia nigra pars reticulata neurons: $n = 12$; $F(2,20) = 3.42$; $P < 0.01$. Because of this effect, post-stimulation data were analyzed as percentages of pre-stimulation firing rate at each dose. Bicuculline dose-dependently reversed the stimulation-evoked inhibition in the substantia nigra pars reticulata: Fig. 4, $n = 12$; $F(1,13) = 6.01$; $P < 0.05$. Post-hoc analysis revealed that this effect was significant at 0.5 mg/kg bicuculline ($P < 0.05$).

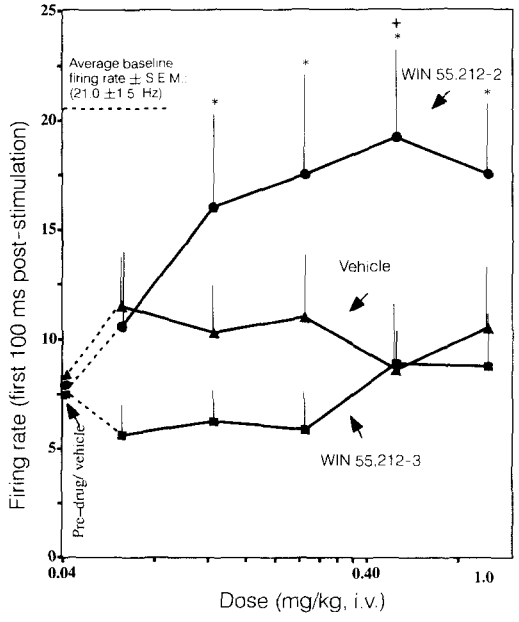

Fig. 3. Effect of WIN 55,212-2 on striatal stimulation-evoked inhibition in the substantia nigra pars reticulata. WIN 55,212-2 dose-dependently reversed the stimulation-evoked inhibition in the substantia nigra pars reticulata ($n = 24$, $P < 0.05$; $^{+}$ significantly different from vehicle; * significantly different from the inactive enantiomer, WIN 55,212-3).

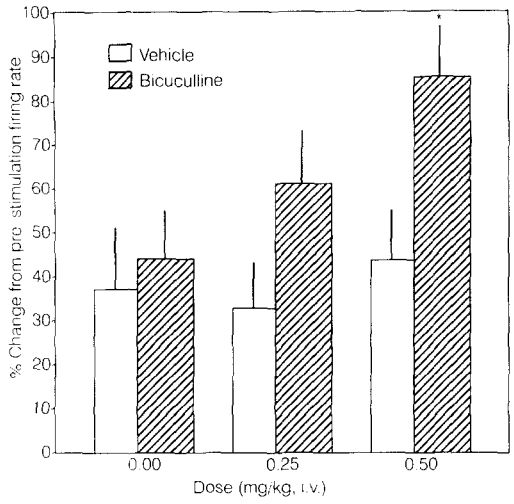

Fig. 4. Effect of bicuculline on striatal stimulation-evoked inhibition in the substantia nigra pars reticulata. Bicuculline dose-dependently reversed the stimulation-evoked inhibition in the substantia nigra pars reticulata ($n = 12$, $P < 0.05$; * $P < 0.05$).

4. Discussion

Systemic injection of a cannabinoid receptor agonist produced a modest increase in the spontaneous firing rate of neurons in the substantia nigra pars reticulata. Because cannabinoid receptors in this area are located on striatonigral terminals, and because cannabinoids inhibit N-type Ca^{2+} channels in NG108-15 cells, we hypothesized that cannabinoids might attenuate the predominantly inhibitory effect of striatonigral neurotransmission. Therefore, the striatum was electrically stimulated while extracellular activity was recorded in the substantia nigra pars reticulata. WIN 55,212-2 dose-dependently attenuated the inhibition of neural activity in the substantia nigra pars reticulata produced by striatal stimulation, whereas neither the vehicle nor the receptor-inactive enantiomer WIN 55,212-3 produced any effect. The lack of pharmacological activity in WIN 55,212-3 in these experiments strongly suggests that the effects we observed were due to specific actions at cannabinoid receptors. These results suggest that one means by which endogenous cannabinoids may regulate movement is by inhibiting striatonigral neurotransmission and thereby disinhibiting the activity of substantia nigra pars reticulata neurons.

Since WIN 55,212-2 increased the spontaneous firing rate of substantia nigra pars reticulata neurons

following either intravenous or intraperitoneal injections, it was surprising that this effect was not observed in the stimulation experiment. Apparently, repetitive stimulation of the caudate altered the physiology of the circuitry in a manner that inhibited the effect of cannabinoids observed with spontaneous firing. The nature of this change is uncertain. Repetitive stimulation may have reduced the spontaneous inhibitory influence of the striatonigral pathway in the substantia nigra pars reticulata, and thereby prevented the disinhibitory effect of cannabinoids hypothesized above. However, further study is needed clarify this issue because the stimulation procedures we used may have activated circuits outside the striatum, and thus other possibilities cannot be excluded.

The possibility that the inhibition observed following electrical stimulation was mediated by GABA was examined in a separate experiment. Previous work demonstrated that the striatum phasically inhibits activity in the substantia nigra pars reticulata by releasing GABA from the striatonigral terminals (Dray et al., 1976; Precht and Yoshida, 1971). Dray et al. (1976) reported that the brief inhibition of firing in the substantia nigra pars reticulata produced by striatal stimulation could be reversed by local administration of the $GABA_A$ antagonist bicuculline. A similar result using our stimulation procedures indicates that GABA-mediated inhibition of substantia nigra pars reticulata neurons can be attenuated by cannabinoids. These findings thus suggest the possibility that cannabinoids inhibit the release of GABA from striatonigral neuron terminals. This is consistent with the small increase in spontaneous firing produced by cannabinoids, because striatal neurons do not produce a profound inhibitory drive on the substantia nigra pars reticulata neurons in anesthetized animals (Grome and McCulloch, 1981).

Although the results of the stimulation experiments and the localization of cannabinoid receptors on striatonigral terminals support the hypothesis that cannabinoids inhibit the release of GABA from striatonigral terminals, it must be emphasized that further work is needed to demonstrate that this occurs. The drugs in these experiments were administered systemically; therefore, it is not possible to state with certainty the site of action of WIN 55,212-2. Other possible explanations for the observed effect include indirect effects of cannabinoid receptors in the striatum, globus pallidus, or subthalamic nucleus. For example, cannabinoid receptors in the globus pallidus mediating an increase of GABA release into this region might indirectly produce an increase of neuronal activity in the substantia nigra pars reticulata by disinhibiting subthalamic neurons which send an excitatory projection to the substantia nigra pars reticulata.

The discovery of a G-protein coupled cannabinoid receptor (Devane et al., 1988) and putative endogenous ligands (Devane et al., 1992; Evans et al., 1992, 1994) raises the question of the functional role of endogenous cannabinoids in the brain. When viewed in the context of the literature on the functional role of the substantia nigra pars reticulata, our results suggest that endogenous cannabinoids may act in the substantia nigra pars reticulata to regulate motor activity and orienting behavior. This hypothesis is supported by demonstrations that the substantia nigra pars reticulata exerts inhibitory control over lower motor structures (Graybiel and Ragsdale, 1979; Niijima and Yoshida, 1982). For example, the substantia nigra pars reticulata exerts inhibitory control over predorsal bundle neurons in the superior colliculus that play an important role in the initiation of orienting movements (Bickford and Hall, 1992). Furthermore, electrical stimulation of the caudate nucleus induces saccadic eye movements and head turning in the cat (Forman and Ward, 1957; Kitama et al., 1991), again suggesting a role of the striatum in orienting behavior. In light of our findings, our data suggest the possibility that cannabinoid inhibition of striatal output circuitry would regulate head and eye movements associated with orienting reactions.

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References

Abood, M.E. and B.R. Martin, 1992, Neurobiology of marijuana abuse, Trends Pharmacol. Sci. 13, 201.

Bickford, M.E. and W.C. Hall, 1992, The nigral projection to predorsal bundle cells in the superior colliculus of the rat, J. Comp. Neurol. 319, 11.

Bidaut-Russell, M., W.A. Devane and A.C. Howlett, 1990, Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain, J. Neurochem. 55, 21.

Bunney, B.S., J.R. Walters, R.H. Roth and G.K. Aghajanian, 1973, Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity, J. Pharmacol. Exp. Ther. 185, 560.

Compton, D.R., K.C. Rice, B.R. De Costa, R.K. Razdan, L.S. Melvin, M.R. Johnson and B.R. Martin, 1993, Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities, J. Pharmacol. Exp. Ther. 286, 218.

Crossman, A.R., R.J. Walker and G.N. Woodruff, 1973, Picrotoxin antagonism of γ-aminobutyric acid inhibitory responses and synaptic inhibition in the substantia nigra, Br. J. Pharmacol. 49, 696.

D'Ambra, T.E., K.G. Estep, M.R. Bell, M.A. Eissenstat, K.A. Josef, S.J. Ward, D.A. Haycock, E.R. Baizman, F.M. Casiano, N.C. Beglin, S.M. Chippari, J.D. Grego, R.K. Kullnig and G.T. Daley, 1992, Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor, J. Med. Chem. 35, 124.

Deniau, J.M., C. Hammond, A. Riszk and J. Feger, 1978, Electrophysiological properties of identified output neurons of the rat substantia nigra (pars compacta and pars reticulata): evidence for the existence of branched neurons, Exp. Brain Res. 32, 409.

Devane, W.A., F.A. Dysarz, M.R. Johnson, L.S. Melvin and A.C. Howlett, 1988, Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol. 34, 605.

Devane, W.A., L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger and R. Mechoulam, 1992, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258, 1946.

Dewey, W.L., 1986, Cannabinoid pharmacology, Pharmacol. Rev. 38, 151.

Dooley, D.J., A. Lupp and G. Hertting, 1987, Inhibition of central neurotransmitter release by ω-conotoxin GVIA, a peptide modulator of the N-type coltage-sensitive calcium channel, Naunyn-Schmied. Arch. Pharmacol. 336, 467.

Dray, A., T.J. Gonye and N.R. Oakley, 1976, Caudate stimulation and substantia nigra activity in the rat, J. Physiol. 259, 825.

Evans, D.M., M.R. Johnson and A.C. Howlett, 1992, Ca^{2+}-dependent release from rat brain of cannabinoid receptor binding activity, J. Neurochem. 58, 780.

Evans, D.M., J.T. Lake, M.R. Johnson and A.C. Howlett, 1994, Endogenous cannabinoid receptor binding activity released from rat brain slices by depolarization, Pharmacol. Exp. Ther. 268, 1271.

Fallon, J.H., F.M. Leslie and R.I. Cone, 1985, Dynorphin-containing pathways in the substantia nigra and ventral tegmentum: a double labeling study using combined immunofluorescence and retrograde tracing, Neuropeptides 5, 441.

Forman, D. and J.W. Ward, 1957, Responses to electrical stimulation of caudate nucleus in cats in chronic experiments, J. Neurophysiol. 20, 230.

Garriott, J.C., L.J. King, R.B. Forney and F.W. Hughes, 1967, Effects of some tetrahydrocannabinols on hexobarbital sleeping time and amphetamine induced hyperactivity in mice, Life Sci. 6, 2119.

Glass, M., R.L. Faull and M. Dragunow, 1993, Loss of cannabinoid receptors in the substantia nigra in Huntingdon's disease, Neuroscience 56, 523.

Gough, A.L. and J.E. Olley, 1978, Catalepsy induced by intrastriatal injections of Δ^9-THC and 11-OH-Δ^9-THC in the rat, Neuropharmacology 17, 137.

Grace, A.A. and B.S. Bunney, 1979, Paradoxical excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons, Eur. J. Pharmacol. 59, 211.

Grace, A.A., D.W. Hommer and B.S. Bunney, 1980, Peripheral and striatal influences on nigral dopamine cells: mediation by reticulata neurons, Brain Res. Bull. 5, 105.

Graybiel, A.M. and C.W. Ragsdale, 1979, Fiber connections of the basal ganglia, Prog. Brain Res. 51, 239.

Grome, J.J. and J. McCulloch, 1981, The effects of chloral hydrate anesthesia on the metabolic response in the substantia nigra to apomorphine, Brain Res. 214, 223.

Grunfeld, Y. and H. Edery, 1969, Psychopharmacological activity of the active constituents of hashish and some related cannabinoids, Psychopharmacologia 14, 200.

Herkenham, M., A.B. Lynn, B.R. De Costa and E.K. Richfield, 1991a, Neuronal localization of cannabinoid receptors in the basal ganglia of the rat, Brain Res. 547, 267.

Herkenham, M., A.B. Lynn, M.R. Johnson, L.S. Melvin, B.R. De Costa and K.C. Rice, 1991b, Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study, J. Neurosci. 11, 563.

Holtzman, D., R.A. Lovell, J.J. Jaffee and D.X. Freedman, 1969, 1-Δ^9-tetrahydrocannabinol: neurochemical and behavioral effects in the mouse, Science 163, 1464.

Howlett, A.C., M.R. Johnson, L.S. Melvin and G.M. Milne, 1987, Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model, Mol. Pharmacol. 33, 297.

Jansen, E.M., D.A. Haycock, S.A. Ward and V.S. Seybold, 1992, Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles, Brain Res. 575, 93.

Khachaturian, H., S.J. Watson, M.E. Lewis, D. Coy, A. Goldstein and H. Akil, 1982, Dynorphin immunocytochemistry in the rat central nervous system, Peptides 3, 941.

Kitama, T., T. Ohno, M. Tanaka, H. Tsubokawa and K. Yoshida, 1991, Stimulation of the caudate nucleus induces contraversive saccadic eye movements as well as head turning in the cat, Neurosci. Res. 12, 287.

Mackie, K. and B. Hille, 1992, Cannabinoids inhibit N-type calcium channels in neuroblastoma glioma cells, Proc. Natl. Acad. Sci. USA 89, 3825.

Mailleux, P. and J.J. Vanderhaeghen, 1992, Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry, Neuroscience 48, 655.

Martin, B.R., 1986, Cellular effects of cannabinoids, Pharmacol. Rev. 38, 45.

Matsuda, L.A., T.I. Bonner and S.J. Lolait, 1993, Localization of cannabinoid receptor mRNA in rat brain, J. Comp. Neurol. 327, 535.

Miller, R.J., 1987, Multiple calcium channels and neuronal function, Science 232, 46.

Niijima, K. and M. Yoshida, 1982, Electrophysiological evidence for branching nigral projections to pontine reticular formation, superior colliculus and thalamus, Brain Res. 239, 279.

Pertwee, R.G. and A.P. Wickens, 1991, Enhancement by chlordiazepoxide of catalepsy induced in rats by intravenous or intrapallidal injections of enantiomeric cannabinoids, Neuropharmacology 30, 237.

Precht, W. and M. Yoshida, 1971, Blockage of caudate-evoked inhibition of neurons in the substantia nigra by picrotoxin, Brain Res. 32, 229.

Vincent, S.R., T. Hökfelt, I. Christensson and L. Terenius, 1982a, Dynorphin-immunoreactive neurons in the central nervous system of the rat, Neurosci. Lett. 33, 185.

Vincent, S.R., T. Hökfelt, I. Christensson and L. Terenius, 1982b, Immunohistochemical evidence for a dynorphin-immunoreactive striato-nigral pathway, Eur. J. Pharmacol. 85, 251.

Waszczak, B.L., 1990, Differential effects of D_1 and D_2 dopamine receptor agonists on substantia nigra pars reticulata neurons, Brain Res. 513, 125.