Role of the Subthalamic Nucleus in Cannabinoid Actions in the Substantia Nigra of the Rat

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Sañudo-Peña, M. Clara and J. Michael Walker. Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. *J. Neurophysiol.* 77: 1635–1638, 1997. The effect of cannabinoids on the excitatory input to the substantia nigra reticulata (SNr) from the subthalamic nucleus was explored. For this purpose a knife cut was performed rostral to the subthalamic nucleus to isolate the subthalamic nucleus and the SNr from the striatum, a major source of cannabinoid receptors to the SNr. The data showed that the cannabinoid agonist WIN55,212-2 blocked the increase in the firing rate of SNr neurons induced by stimulation of the subthalamic nucleus with bicuculline. Furthermore, the cannabinoid antagonist SR141716A antagonized the effect of the cannabinoid agonist. This study showed that cannabinoids regulate not only the striatonigral pathway, as previously reported, but also the subthalamonigral pathway. The opposite influences of these two inputs to the SNr, inhibitory and excitatory respectively, suggest that endogenous cannabinoids play a major role in the physiological regulation of the SNr.

INTRODUCTION

The term cannabinoid refers to psychoactive compounds obtained from the plant *Cannabis sativa* (marijuana) and to other exogenous and endogenous substances that possess a similar pharmacological profile. Other than their well-known psychotropic actions, these substances induce many different effects including profound changes in motor function (Abood and Martin 1992; Howlett 1995). The motor effects of cannabinoids are not surprising when one considers the high levels of cannabinoid receptors in brain areas implicated in the control of movement (Herkenham et al. 1991a,b; Mailleux and Vanderhaeghen 1992). The basal ganglia, a group of brain nuclei involved in motor processing, possess particularly high levels of cannabinoid receptors (Herkenham et al. 1991a,b; Mailleux and Vanderhaeghen 1992).

The substantia nigra pars reticulata (SNr), an output structure for the basal ganglia, has extremely high levels of cannabinoid receptors (Graybiel 1990; Herkenham et al. 1991a,b; Mailleux and Vanderhaeghen 1992). SNr neurons secrete γ-aminobutyric acid (GABA), tonically inhibiting movement production through the inhibition of their projection areas (reviewed by Albin et al. 1989; DeLong 1990; Graybiel 1990). However, the substantia nigra is devoid of detectable levels of mRNA for the cannabinoid receptor, which indicates that the receptors are mainly located on axon terminals in this structure (Herkenham et al. 1991a; Mailleux and Vanderhaeghen 1992). The striatum has been identified as a major source of cannabinoid receptors to the substantia nigra, because lesions of the striatum eliminate most of the cannabinoid receptor binding in this area (Herkenham et al. 1991a).

Cannabinoids inhibit neurotransmission through activation of K^+ channels and inhibition of N- and Q-type voltage-gated calcium channels. These effects are mediated through G proteins (Deadwyler et al. 1993; Howlett 1995; Mackie and Hille 1992; Mackie et al. 1995). Also, inhibition of voltage-gated Na^+ channels by cannabinoids has been reported (Turkanis et al. 1991). Furthermore, cannabinoids inhibit the release of various neurotransmitters including GABA and glutamate (Revuelta et al. 1982; Shen et al. 1996). In accordance with these data, cannabinoids blocked the inhibitory action of the striatal input to the substantia nigra (Miller and Walker 1995). Similarly, intranigral administration of cannabinoids reversed the contralateral rotation induced by dopamine D1 agonists microinjected into the SNr that act at striatonigral terminals releasing GABA and dynorphin (Sañudo-Peña et al. 1996; You et al. 1994). This finding again suggested that cannabinoids inhibit neurotransmitter release in the SNr. However, the administration of cannabinoid agonists alone in the SNr induced contralateral rotation, a finding consistent with cellular inhibition in this circuitry and therefore opposite to the facilitation that would be expected from a cannabinoid action at striatonigral terminals in the SNr (Di Chiara et al. 1979; Sañudo-Peña et al. 1996). This apparent contradiction suggested the presence of a second site of action of cannabinoids in the SNr that may mediate these unexpected behavioral effects of cannabinoids (Sañudo-Peña et al. 1996).

Whereas the striatonigral input is inhibitory, the subthalamic nucleus provides the main excitatory drive to the SNr (Graybiel 1990; Robledo and Feber 1990). Therefore it represents a good candidate for an opposite action of cannabinoids within this circuitry. The current work was designed to test this hypothesis.

METHODS

Male Spague-Dawley rats (Charles River Laboratories), ~280–320 g at the time of surgery, served as subjects. On the day of the experiment the animals were anesthetized with chloral hydrate (initial intraperitoneal injection of 400 mg/kg, followed by intravenous supplemental administration as needed) and placed in a stereotaxic frame. A glass cannula (142 μm OD, Polymicro Technologies, Phoenix, AZ) was implanted into the subthalamic nucleus (coordinates: 5.2 mm anterior, 12.2 mm lateral, 13.1 mm ventral from bregma and the skull surface with a 50° angle) and the subthalamic nucleus was stimulated by injection of 0.2–0.4 μl of 0.05 μg/μl bicuculline methiodide (RBI, Natick, MA). Because the
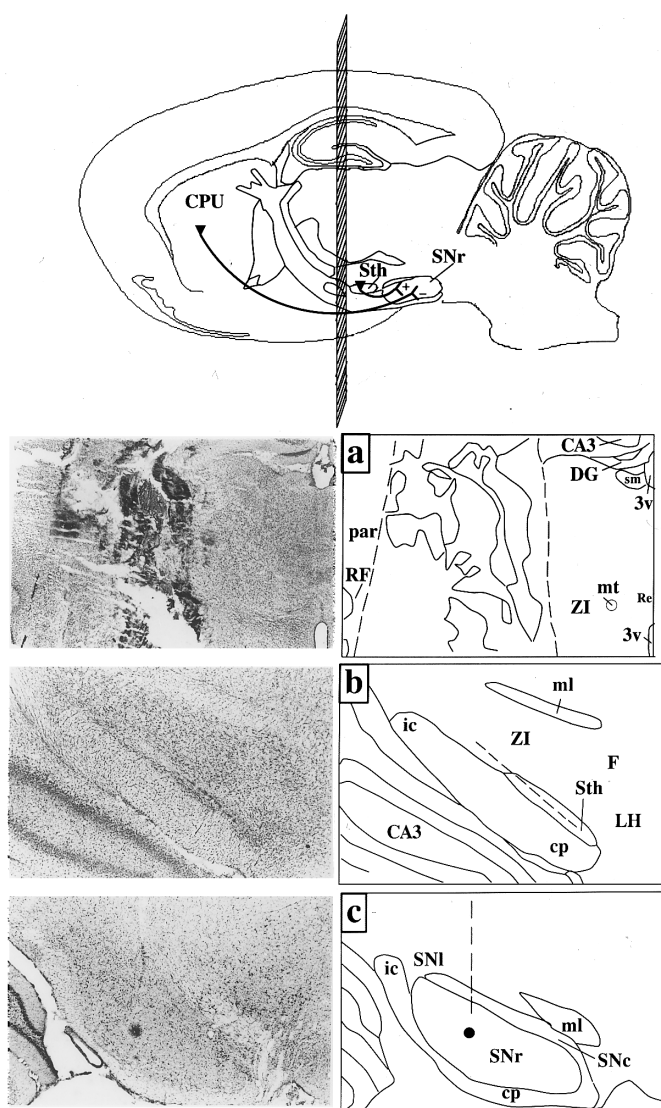
FIG. 1. Schematic of the preparation employed in this study. *Top*: drawing of the rat brain in sagittal section showing the known inputs to the substantia nigra pars reticulata (SNr) that contain cannabinoid receptors and the level at which the knife cut was performed (hatched vertical plane). The striatonigral pathway is inhibitory, whereas the subthalamonigral input is excitatory. CPU, caudate-putamen; Sth, subthalamic nucleus. *a, left*: photograph coronal section of rat brain showing location and extent of the knife cut rostral to the Sth demarcated by the dashed lines in the schematic at *right*. CA3, field CA3 of Ammon's horn; DG, dentate gyrus; mt, mammillothalamic tract; par, parietal cortex; Re, reuniens thalamic nucleus; RF, rhinal fissure; sm, stria medullaris of the thalamus; ZI, zona incerta; 3v, 3rd ventricle. *b, left*: photograph coronal section of rat brain at the level of the caudal diencephalon showing tract (dark area) of the glass cannula in the subthalamic nucleus represented by the dashed line in the schematic at *right*. cp, cerebral peduncle; F, nucleus of the fields of Forel; ic, internal capsule; LH, lateral hypothalamic area; ml, medial lemniscus. *c, left*: photograph of a coronal section of rat midbrain showing tract of microelectrode represented by the dashed line in the schematic at *right*, with the tip represented by the black circle in the SNr. SNc, substantia nigra compacta; SNl, substantia nigra lateral.

subthalamic nucleus also innervates the striatum and globus pallidus, which in turn project to the SNr, a knife cut was performed 1 mm rostral to the location of the cannula in the subthalamic nucleus to isolate these rostral areas of the basal ganglia from the subthalamic nucleus and substantia nigra (see Fig. 1). The lesion

also disrupts the striatonigral pathway, an assumed site of action of cannabinoids at the SNr. A stainless steel microelectrode (impedance 5 MΩ; FHC, Brunswick, ME) was stereotaxically inserted into the SNr with the aid of a hydraulic device. Coordinates were 3.2 mm anterior, 2.2 mm lateral, 6.7–8.7 mm ventral from lambda and the skull surface. SNr cells were identified by their characteristic firing properties (Bunney et al. 1973) and by their location ventral to dopaminergic cells in the SNc. At the end of the experiment the location was marked by passing 2 mA of current for 15–30 s through the electrode. The sections were fixed with 10% Formalin with 1% potassium ferrocyanide to mark the location of metal deposit. The cannabinoid agonist WIN55,212-2 (0.5 mg/ml) and the antagonist SR141716A (0.5 mg/ml) were dissolved in 25% 2-hydroxypropyl-β-cyclodextrin (RBI, Natick, MA) and administered intravenously at a dose of 1 mg/kg. Data analysis was performed with the use of the paired *t*-test.

RESULTS

Histological examination of the tissue revealed that the knife cut effectively separated the substantia nigra and subthalamic nucleus from rostral areas of the brain (Fig. 1, *top*). Furthermore, the microinjection in the subthalamic nucleus was verified in each animal included in the study and was found to occur with minimal damage to the surrounding tissue (Fig. 1*b*). Examination of the metal deposits placed at the end of each experiment verified that all recording sites were localized to the SNr (Fig. 1*c*).

As observed previously (Bunney et al. 1973), SNr neurons ($n = 10$) exhibited brief (<1 ms) action potentials that occurred spontaneously at a rate of 51 ± 5 (SE) Hz, range 30–70 Hz. All of the neurons we recorded showed an even rate of discharge. Microinfusion of bicuculline into the subthalamic nucleus induced a significant increase in the firing rate of SNr neurons ($20 \pm 4\%$, range 7–40%, $n = 10$; $P \leq 0.002$, Fig. 2*a*, Table 1), as reported previously in intact animals (Robledo and Feber 1990). This increase in firing was attenuated by the the cannabinoid agonist WIN55,212-2 ($P \leq 0.005$, Fig. 2*b*, Table 1). The effect of WIN55,212-2 was significantly antagonized by the competitive cannabinoid antagonist SR141716A ($P \leq 0.006$).

DISCUSSION

The experiments described above demonstrate that cannabinoids dampen the excitatory drive in the SNr produced by the subthalamic nucleus. This effect was apparently mediated by an action of the drug at cannabinoid receptors, because it was antagonized by SR141716A. Such a conclusion is consistent with the existence of cannabinoid receptor mRNA in the subthalamic nucleus (Mailleux and Vanderhaeghen 1992) and data suggesting that cannabinoids typically inhibit neurotransmission (Deadwyler et al. 1993; Howlett 1995; Mackie and Hille 1992; Mackie et al. 1995; Turkanis et al. 1991). The fact that cannabinoids also appear to inhibit neurotransmission at the striatal input to the SNr (Miller and Walker 1995; Sañudo-Peña et al. 1996) suggests that cannabinoids modulate both inhibitory and excitatory inputs to the SNr and may thus play a major role in the regulation of this important output structure of the basal ganglia.

The lesion performed in this study isolated the subthalamonigral pathway from rostral regions of the brain, leaving
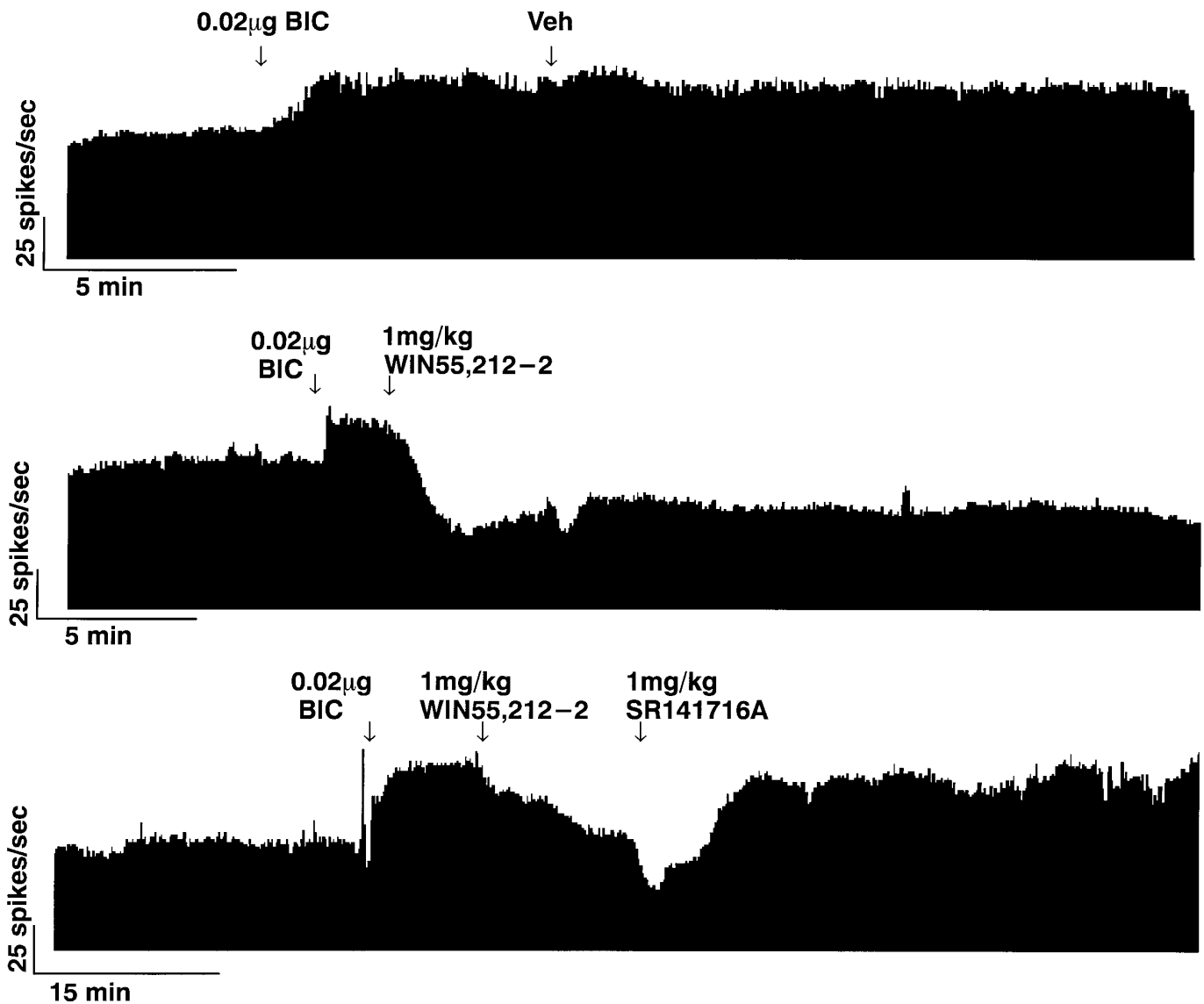
FIG. 2. Firing rate histograms obtained from 3 different neurons as follows. *a*: microinjection of bicuculline (BIC) (0.01–0.02 μg/0.2–0.4 μl) in the subthalamic nucleus caused long-lasting excitation of SNr neurons. *b*: WIN55,212-2 (1 mg/kg iv) significantly attenuated this effect. *c*: SR141716A antagonized the effect of WIN55,212-2. The short-lasting decrease in the firing rate apparent immediately following administration of the antagonist could not be attributed to the drug, because similar short-lasting decreases were seen in other animals in the absence of the antagonist.

the SNr devoid of several possible afferent sources of cannabinoid receptors other than the subthalamic input. Indeed, this lesion left the subthalamic nucleus with no known direct

TABLE 1. *Mean firing rates of SNr neurons*

Baseline, Hz	Subthalamic bicuculline, Hz	WIN 55,212-2, Hz	SR141716A, Hz/% reversal
49 ± 5	63 ± 9*	36 ± 7†	60 ± 14/81 ± 11‡

Values are means ± SE of firing during baseline, subthalamic nucleus stimulation, and after administration of the cannabinoid agonist WIN55,212-2 (iv) and the cannabinoid antagonist SR141716A (iv).
* Significantly different from baseline, $P \leq 0.03$.
† Significantly different from subthalamic bicuculline, $P \leq 0.005$.
‡ Significant antagonism, $P \leq 0.006$.

or indirect afferent system containing cannabinoid receptors (Mailleux and Vanderhaeghen 1992; Zahm and Alheid 1994). Therefore it appears that the cannabinoids blocked the increase in firing of SNr neurons produced by subthalamic nucleus stimulation with bicuculline by a direct action on the subthalamonigral pathway.

The recent report that cannabinoids inhibited presynaptic glutamate release in hippocampal neurons, while not affecting the presynaptic resting membrane potential, action potential threshold, action potential duration, or action potential amplitude, suggests an important role of cannabinoids in inhibiting neurotransmitter release (Shen et al. 1996). Because of this and a previous report demonstrating inhibition of N- and Q-type Ca^{2+} channels by cannabinoids (Mackie and Hille 1992; Mackie et al. 1995), we are inclined to interpret the effects of cannabinoids in this study as an inhi-

bition of glutamate release from the subthalamic terminals in the SNr. Any hyperpolarizing action of cannabinoids at the subthalamonigral pathway might be expected to be masked by the large depolarizing effect of the GABAergic antagonist applied at the subthalamic nucleus. For these reasons it appears likely that the cannabinoid exerted its effects at subthalamic terminals in the SNr.

The conclusion that the cannabinoid acted at subthalamic nucleus terminals would agree with the previous observation of contralateral rotational behavior following cannabinoid microinjections in the SNr (Sañudo-Peña et al. 1996). Inhibition of excitatory neurotransmission from the subthalamic nucleus by a nigral microinjection of a cannabinoid would be expected to lead to contralateral rotational behavior, because inhibition of SNr neurons leads to disinhibition of movement (Di Chiara et al. 1979; Graybiel 1990; You et al. 1994). Behavioral effects mediated by drug action at SNr terminals would mainly reflect the actions on the subthalamic input, because it is tonically active, whereas the inhibitory input from the striatum is quiescent (Robledo and Feber 1990; Wilson 1993).

The subthalamic nucleus plays an important role in motor disorders. Hyperactivity of the subthalamic nucleus is thought to be associated with some types of akinesias, such as that characteristic of Parkinson's disease (Albin et al 1989; DeLong 1990). The present study demonstrates that a cannabinoid agonist blocked the experimentally induced activation of the subthalamic nucleus input to the SNr, probably by its ability to inhibit neurotransmitter release at these terminals. These observations may point to a therapeutic role for cannabinomimetic drugs in the treatment of movement disorders.

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