



Pergamon

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

NEURO
PHARMACOLOGY

Neuropharmacology 44 (2003) 633–641

www.elsevier.com/locate/neuropharm

Differential cannabinoid-induced electrophysiological effects in rat ventral tegmentum

J.F. Cheer¹, D.A. Kendall, R. Mason, C.A. Marsden*

School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

Received 19 July 2002; received in revised form 18 December 2002; accepted 23 December 2002

Abstract

Cannabinoids are known to exert mainly excitatory effects on dopaminergic cells of the ventral tegmental area (VTA). We have utilized an in vivo multiple-single unit electrophysiological approach to assess different neuronal contributions that may ultimately lead to excitation in this area. Baseline neuron recordings, using low impedance microwires, showed a variety of waveforms with a wide range of durations (0.8–3.2 ms). In the first experiment systemic injection of the potent cannabinoid agonist HU210 (100 µg/kg, i.p.) led predominantly to an increase in firing rate (~214%, compared to pre-drug) in slowly firing cells with broad action potentials, possibly driven by a majority of presumed dopaminergic neurons ($n = 31$). However, the firing rate of some units was either unaffected ($< 25%$, $n = 9$) or even decreased (~67%, $n = 9$) following cannabinoid injection concomitantly with excitation. Apomorphine (75 µg/kg, i.p.) injected following HU210 produced a marked inhibition of both responses (~76%) in 39 out of 49 cells. The second group of animals was treated with the CB₁ receptor antagonist SR141716A (1 mg/kg, i.p.), which had no effect when injected alone but prevented all HU210-evoked changes in firing rate suggesting that cannabinoid receptors mediated the observed responses ($n = 39$). Taken together, the present results suggest that the observed actions of cannabinoids may involve complex neurotransmitter interactions leading to differential effects on dopamine release. These heterogeneous neuronal responses are likely to underly the behavioural discrepancies reported in animal models of cannabinoid reinforcement.
© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: HU210; Reinforcement; Ensemble recording; Dopamine; VTA

1. Introduction

Although humans have used marijuana since ancient times for recreational purposes (Mechoulam, 1986), the rewarding properties of cannabinoid compounds have been difficult to demonstrate experimentally in animal models, in spite of increasing evidence in favor of mechanistic effects for these compounds in brain areas related to reinforcement (for review see Schlicker and Kathmann, 2001). Cannabinoids, such as Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychoactive ingredient in the marijuana plant, have been shown to induce conditioned place preference (CPP) (Braida et al., 2001a),

to lower the threshold for electrical self-stimulation in rats (Gardner et al., 1988) and to be self-administered intravenously (Fattore et al., 2001) and intracerebroventricularly in rats (Braida et al., 2001b) as well as intravenously in mice (Martelotta et al., 1998). In a primate model (squirrel monkey), low doses of Δ^9 -THC have also been shown to be self-administered intravenously following cocaine pre-exposure (Tanda et al., 2000). Nevertheless, synthetic cannabinoids as well as Δ^9 -THC have also been reported to elicit negative motivational states. These compounds may produce conditioned place aversion (Cheer et al., 2000a), have anxiogenic properties in the elevated plus maze (Arevalo et al., 2001) and are not self-administered by rhesus monkeys (Mansbach et al., 1994).

The mesocorticolimbic dopamine (DA) system has been implicated in reward behavior and drug dependence (for review see Self and Nestler, 1995). Dopaminergic neurons of this pathway originate in the ventral tegmental area (VTA) and project to limbic and fronto-cortical

* Corresponding author. Tel.: +44-0-115-970-9480; fax: +44-0-115-970-9259.

E-mail address: charles.marsden@nottingham.ac.uk (C.A. Marsden).

¹ Current address: Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

Nomenclature

5-HT, serotonin	5-hydroxytryptamine
CB ₁	central cannabinoid receptor
CPP	conditioned place preference
DA, dopamine	3-hydroxytyramine
Δ ⁹ -THC	Δ ⁹ -tetrahydrocannabinol
HU210	(-)-11-hydroxy-Δ ⁸ -tetrahydrocannabinol-dimethyl-heptyl
JFET	J-type field effect transistor
MAP	multi-neuron acquisition processor
NAC	Nucleus Accumbens
NEX	NeuroExplorer
SR141716A, SR	<i>N</i> -piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide
TH	tyrosine hydroxylase
VTA	Ventral Tegmental Area

areas such as the nucleus accumbens (NAc). Increases in VTA dopaminergic cell firing show an apparent lack of desensitization when assessed *in vitro* (Cheer et al., 2000b) or *in vivo* (Wu and French, 2000). In agreement with electrophysiological data, cannabinoids have also been shown to increase dopamine release in the NAc (Tanda et al., 1997) or locally in the VTA (Chen et al., 1993).

The aim of the present investigation was to further our current understanding of the neurobiological basis of cannabinoid effects on motivation and reward by studying VTA cell firing dynamics. The main objective of the study was to determine the contribution of different neuronal responses to acute cannabinoid administration assessed by multiple single-unit electrophysiology in the anesthetized rat. We examined the involvement of cannabinoid receptors in these responses by studying the effects of the CB₁ receptor antagonist SR141716A.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats ($n = 15$, Charles River Laboratories, UK), weighing 250–350 g at the time of surgery were housed in groups of three in large plastic cages and maintained on a 12:12 hr light-dark cycle (lights on at 7:00 AM). Water and food were available *ad libitum*. All experiments were performed in accordance to UK Home Office regulations under project license 40/1955.

2.2. Surgical procedure

Animals were initially induced with 4% halothane (a more rapidly-inducing and controllable inhalation

anesthetic) and then mounted on a modified Kopf model 1730 stereotaxic apparatus. Anesthesia was maintained with halothane at 0.75% and supplemented with 70% N₂O : 30% O₂. However, this anesthetic protocol was abandoned following three experiments in which a single injection of the cannabinoid agonist HU210 (40, 60 or 100 μg/kg; *i.p.*) depressed breathing with animals dying shortly thereafter. To avoid this drug interaction, the anesthetic was changed to chloral hydrate (15% w/v in distilled water). Animals were given an initial *i.p.* bolus of 500 mg.kg⁻¹ and then supplemented (100 mg.kg⁻¹; *i.p.*) every hour, corresponding with lighting of level of anesthesia, until the end of the experiment. A 4 x 2 microwire electrode array or a 16 microwire electrode bundle (Teflon-coated stainless steel, 50 μm diameter; NBLabs, TX), connected to 8- or 16-channel JFET headstage (NBLabs, TX) were utilized. Additional stainless steel electrodes present on the electrodes' headsets provided the grounding lead. Microelectrodes were lowered for stereotaxic positioning using a Narishige SM15 micromanipulator (−4.7 to −6.0 mm A/P, 0.3–0.8 L relative to bregma and −7.4 to −7.8 D/V to the brain surface) according to Paxinos and Watson (1986). A square piece of bone (2.5 x 2.5 mm) was excised with the aid of watchmakers' forceps to expose the dura matter under microscopic control. At this stage, the surgical plane was covered with a 5% Marcain® adrenaline solution (Astra, UK), which prevented excessive bleeding (due to the proximity of the stereotaxic co-ordinate to the sagittal sinus and its collateral vessels) when the meningeal membrane was dissected. The local anesthetic solution was exchanged with saline following electrode positioning to prevent the cortical surface from drying out. The temperature of the animal was maintained at 37.7 C with the aid of a rectal probe connected to a homeothermic blanket control unit (Harvard Apparatus, MA).

2.3. Electrophysiological recordings

On-line isolation and discrimination of neuronal spike activity was determined using commercially available hardware and software (Plexon Inc, TX). Briefly, the eight-channel micro J-type field effect transistor (JFET) provided unity-gain current amplification from each microwire in the bundle (the impedance of each microwire was ~ 100 k Ω measured at 1 kHz). A second level of amplification occurred at a 16-channel differential preamplifier (fixed gain $\times 100$), after which the signal was transferred to a Multineuron Acquisition Processor (MAP). The MAP allowed for computer-controlled amplification, filtering (highpassed with a 500 Hz cutoff), switching and digital signal processing of the signals originating from the microwires. Connection to a host PC workstation running under Windows NT was made via a serial line and an external 16-bit parallel MXI bus. A software version of a threshold-level Schmitt trigger was used initially to isolate incoming signals from background noise. Once this was achieved, experimenter-defined time-voltage boxes present on the display window of the acquisition software were used to collect all threshold-spanning waveforms. Typically, one to four neurons were observed per microwire during any given experiment. Activity was also displayed on a Tektronix D11 5000 series dual-beam oscilloscope and corresponding action potential waveforms were isolated with the aid of a Gould 1425 Digital Oscilloscope and a Gould Type 125 waveform processor. The firing rate was also monitored aurally with the aid of a loudspeaker.

2.4. Drugs

(-)-11-hydroxy- Δ^8 -tetrahydrocannabinol-dimethylheptyl (HU210) was obtained from Tocris Cookson (Bristol, UK) and apomorphine were obtained from Sigma Chemical (Gillingham, UK), N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A) was a generous gift from Sanofi Recherche. HU210 was re-suspended to stock concentration (10^{-2} M) in ethanol and refrigerated at -20 C; SR141716A and apomorphine were weighed out on the day of the experiment. HU210 was dissolved in a physiological 1.0 ml saline (0.9% NaCl) 10 μ l Tween 80 (1%) vehicle solution for injection on the day of the experiment, SR141716A was dissolved in a 0.8 ml saline 100 μ l ethanol 100 μ l Tween 80 (1%) vehicle and apomorphine was diluted in physiological saline (0.9%) for injection.

2.5. Data analysis

Neuronal signals were sorted offline via principal component analysis to ensure isolation of the recorded signal. Discriminated action potentials were processed

using NeuroEXplorer (NEX Technologies, NC). Descriptive firing rate histograms were computed from spike data spreadsheets. Basal activity was calculated as the mean \pm SEM of firing over a 5-min pre-drug period for overall firing analyses. The threshold for percent change was set at 25% relative to baseline due to the high variability in basal firing observed between cells (firing rates ranged from 0.71 ± 0.05 to 18.2 ± 2.18 Hz). Numerical results were obtained from NEX and one-way analyses of variance (ANOVA) were used to study treatment response differences followed by Scheffe's post hoc tests. The criterion of significance for the ANOVAs and post hoc tests was set at $P < 0.05$. All statistical analyses were computed using Statistica (Statsoft Inc, OK).

2.6. Histology

On completion of all experiments the location of the recording electrode tips was marked by passing a 60 μ A current for 30 s through selected electrode pairs in the array. Animals were then transcardially perfused with a 4% paraformaldehyde 5% potassium ferrocyanide solution. Ferrocyanide creates a blue-green stain by reacting with deposited iron ions at the mark site. The brains were then removed and stored at 4 C in the same fixative solution overnight. Vibratome sections were then cut (80 μ m) and transferred to slides with the aid of mounting medium (Vectashield), without any further counterstaining, for histological verification.

3. Results

3.1. Duality of HU210-evoked responses in the VTA

Ventral tegmental area recordings were obtained at -7.4 to -7.8 mm from brain surface (Figs. 1 and 5).



Fig. 1. Digitally derived waveforms showing the firing of four simultaneously recorded VTA neurons from two individual microwire electrodes from a 4×2 array. Waveform calibration: 80 μ V, 0.5 ms.

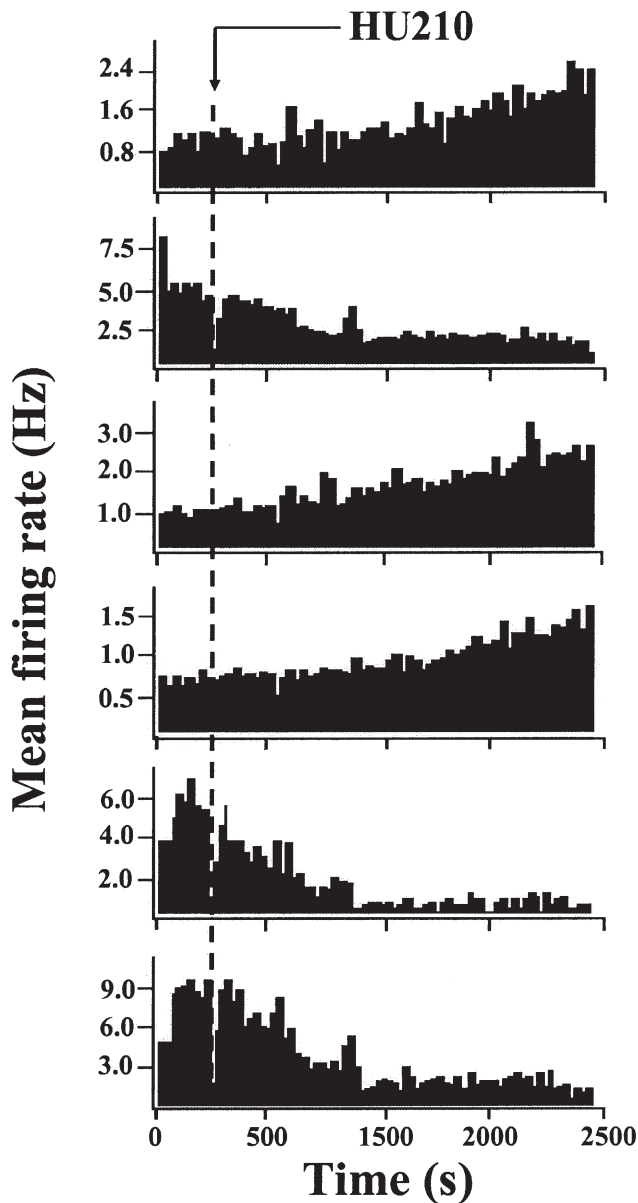


Fig. 2. Integrated firing rate histograms of 6 simultaneously recorded representative neurons showing the excitatory and inhibitory effects of the potent cannabinoid agonist HU210 on VTA neuron baseline firing rate. The prevalent type of excitation was a mild-sloped ramp-up in firing 20 min following the injection, as depicted in the first histogram. The inhibition induced by HU210 was (see histograms 2, 5 and 6) had a more rapid onset compared to the excitatory effects and the magnitude of the change compared to pre-drug firing rate was greater. Arrowhead and dashed line indicate a $100 \mu\text{g.kg}^{-1}$ injection (i.p.). Rate Histograms, bin = 20 s.

There was no consistent relationship between the anatomical location (rostral-caudal or dorso-ventral) and the response characteristics of any given neuron. A total of 49 neurons ($n = 6$ animals) were assessed for the effects of an acute injection of the potent cannabinoid agonist HU210 ($100 \mu\text{g.kg}^{-1}$ i.p.) on VTA neuronal activity. The threshold for considering a treatment-evoked response was set as a 25% change in firing rate, due to the high

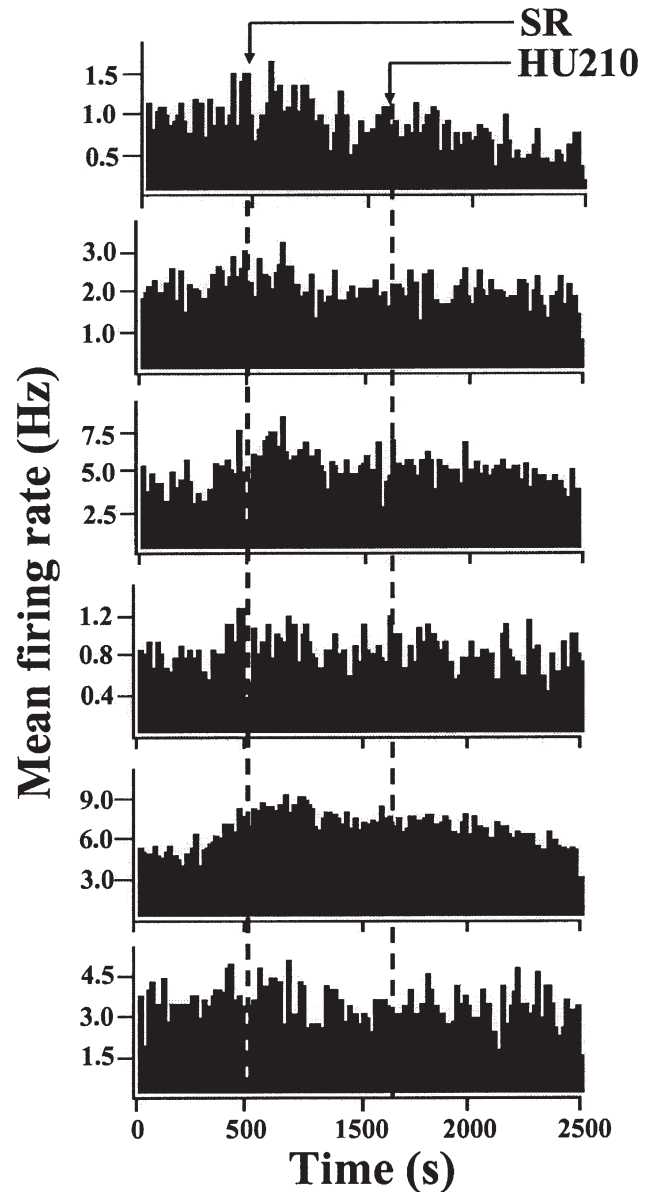


Fig. 3. Integrated firing rate histograms of six simultaneously recorded VTA neurons showing the effects of the potent cannabinoid agonist HU210 injected in the presence of the cannabinoid antagonist SR141716A on basal firing rate. Arrowheads indicate a 1.0 mg.kg^{-1} of SR and a $100 \mu\text{g.kg}^{-1}$ injection of HU210 (both injections: i.p.). When injected in the presence of SR (which had no effect on its own); HU210 did not affect the basal firing rate of VTA neurons. Rate Histograms, bin = 20 s.

variability in baseline firing. Thirty-one out of 49 neurons were significantly excited by the injection of HU210 from 4.2 ± 1.0 Hz (pre-drug basal firing; mean \pm SEM) to 13.2 ± 3.5 Hz (~214% increase in firing, $F_{(2,121)} = 3.14$; $p < 0.01$, Fig. 2) while nine out of 49 neurons were significantly inhibited 8.3 ± 1.7 Hz to 5.6 ± 2.3 Hz (~67% decrease in firing, $F_{(2,121)} = 3.14$; $p < 0.01$, Figs. 2 and 5). Nine of 47 neurons remained unaffected by the cannabinoid treatment; pre-drug basal: 3.5 ± 1.2 Hz and post-drug: 4.3 ± 1.0 Hz (Fig. 5). The

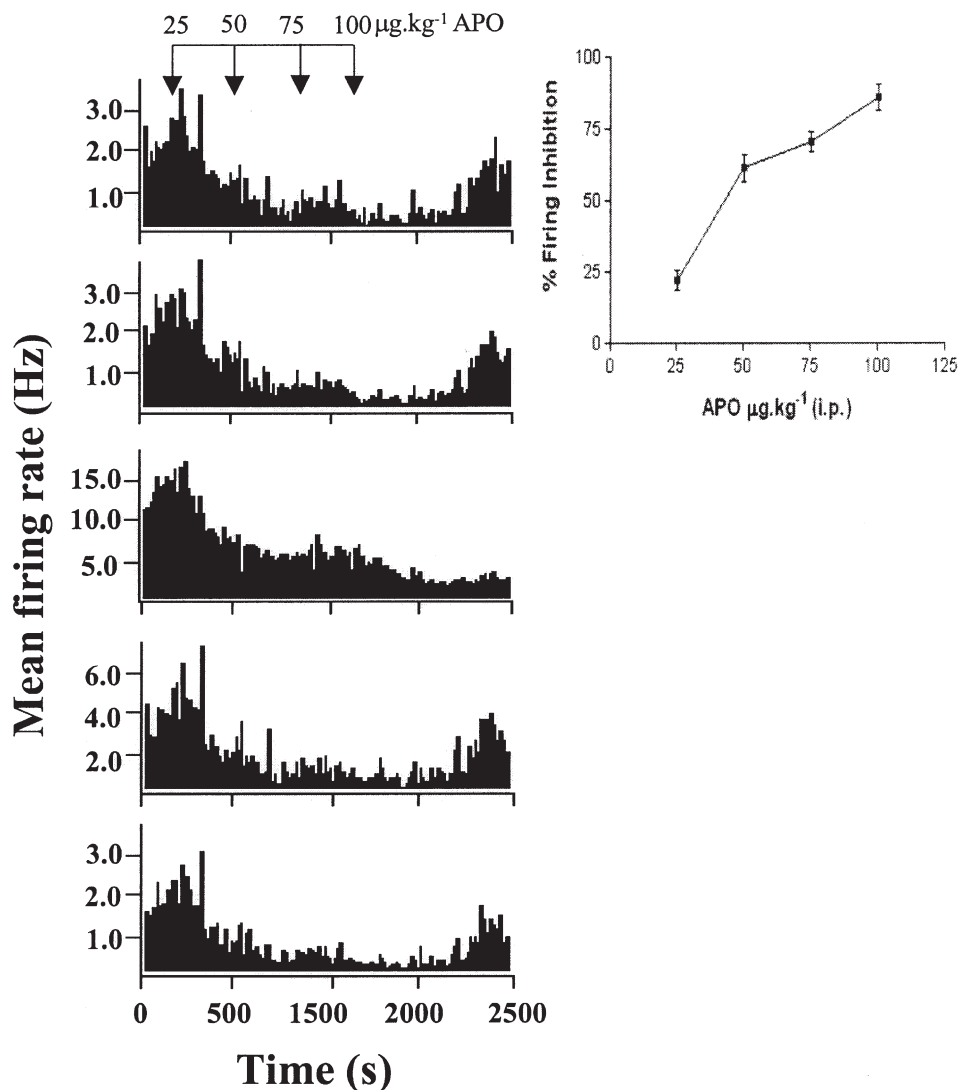


Fig. 4. Integrated firing rate histograms showing a dose-dependent apomorphine-induced decrease in the firing rate of multiple VTA neurons in a naïve animal. Note that the firing rate of neuron three is higher than the rest of the neurons and it is responding differently to the treatment. (inset) Dose-response of apomorphine-induced inhibition for all neurons studied ($\text{ED}_{50} = 40 \mu\text{g}\cdot\text{kg}^{-1}$).

baseline firing rates of cells that were excited by HU210 were significantly lower than the ones that were inhibited but higher than those that were unaffected by HU210 (4.2 ± 1.0 and 8.3 ± 1.7 Hz and 3.5 ± 1.2 Hz respectively; $F_{(2, 121)} = 2.93$, $p < 0.05$). The lag-time for an HU210 injection to have a stable effect was ~ 20 min.

3.2. The cannabinoid antagonist SR141716A prevents HU210-induced changes in VTA neuronal firing

In order to investigate whether HU210 ($100 \mu\text{g}\cdot\text{kg}^{-1}$) was exerting its effects via CB_1 receptors, the CB_1 receptor antagonist SR was assessed at a dose of $1.0 \text{ mg}\cdot\text{kg}^{-1}$ 20 min before HU210 treatment (39 neurons, $n = 6$ rats). The threshold for significant firing rate change was set at 25%. SR injection did not have a significant effect on firing rate when injected on its own (Fig. 3). When

injected in the presence of SR, HU210 failed to excite the basal firing rate of all the studied neurons (39/39). Following HU210 injection, cells showed a trend towards inhibition although this effect did not reach significance (4.6 ± 1.2 Hz to 3.9 ± 0.9 Hz; $\sim 17\%$ $< 25\%$ change in firing rate) (Fig. 3).

3.3. Apomorphine reversal of HU210-induced actions on VTA cells

When given alone to naïve animals (17 neurons, $n = 3$ rats), apomorphine dose-dependently decreased the firing of almost all (12/17) simultaneously recorded neurons ($\text{ED}_{50} \sim 40 \mu\text{g}\cdot\text{kg}^{-1}$) (Figs. 4 and 5). Apomorphine also had a powerful depressant effect on HU210-modified (i.e. enhanced or reduced) VTA activity 35 min after injection (Fig. 5). Out of 49 neurons recorded (same ani-

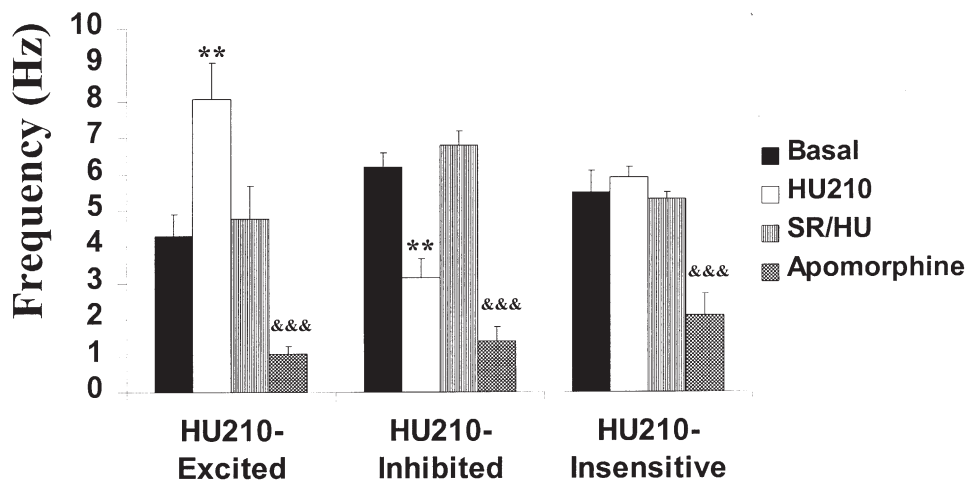


Fig. 5. Bar graph showing the changes in mean baseline firing rate of the three categories of cells observed according to their response to a systemic injection of the high affinity cannabinoid receptor agonist HU210. For each of the treatments used in the study (SR/HU) indicates the effects of HU210 in the presence of the cannabinoid antagonist SR141716A, apomorphine indicates the effects of this drug injected following HU210. Each bar represents mean firing rate \pm SEM. Asterisks indicate $p < 0.01$ of the effect of HU210 on baseline firing rate, “&” indicates $p < 0.001$ of apomorphine on firing rate compared to stable HU210-evoked change from baseline.

mals as in the HU210-alone experiment); 39 cells (27 were excited by HU210, seven were inhibited and five were unaffected) were inhibited following a $75 \mu\text{g}\cdot\text{kg}^{-1}$ injection of apomorphine from 7.1 ± 2.3 Hz (HU210-induced plateau firing for all cells; mean \pm SEM) to 1.7 ± 0.4 Hz ($\sim 76\%$ firing rate inhibition, $F_{(2,121)} = 1.24$; $p < 0.0001$) (Fig. 5). The rest of the neurons were unaffected by apomorphine ($n = 10$) in the presence of HU210. The lag-time for an apomorphine injection to have a stable effect was ~ 15 min.

4. Discussion

The present study shows, for the first time, that the high-potency central cannabinoid receptor agonist HU210 is able to produce differential changes in the firing rates of simultaneously recorded neurons within the VTA. Dopaminergic neuron excitations have been reported following Δ^9 -THC treatment (French, 1997; Melis et al., 2000). Since the VTA contains a majority of DA neurons ($\sim 70\%$; Hökfelt et al., 1980), the excitation of a majority of cells recorded in this study may underly the cannabinoid-enhanced DA release seen in terminal areas such as the NAc (Tanda et al., 1997). Surprisingly, injection of HU210 also elicited decreases in firing rate in a minority of units (19%) and an even smaller number of neurons (17%) were insensitive to the cannabinoid. Taken together these findings point to the existence of more than two different cellular targets of cannabinoids recorded by the microwire electrode assemblies used in the present study. Previous *in vitro* (Cheer et al., 2000b) and *in vivo* (Wu and French, 2000; Melis et al., 2000) experiments have failed to observe a cannabinoid-induced inhibition of VTA neuronal firing,

emphasizing the importance of recording from more than one neuron when assessing the effects of this particular type of compounds. Moreover, the HU210-induced excitations seen in the VTA-containing slice were several orders of magnitude greater than those observed *in vitro* (Cheer et al., 2000b), a difference that may be explained by the deafferentation exhibited by the slice preparation. Taken together, these findings highlight the crucial regulation neurons originating from outside the VTA exert on this particular neuronal population (Floresco et al., 2001).

The VTA is under tonic inhibitory control from the ventral pallidum and the NAc (Tzschenke, 2001). Medium spiny neurons from the NAc which utilize GABA as a neurotransmitter, project to the VTA where they can affect dopaminergic neurons or GABAergic projection neurons (Tzschenke, 2001). Since adult dopaminergic neurons do not express CB_1 receptors (Hernandez et al., 2000), it is likely that HU210 may have interfered with inhibitory inputs to the VTA. This claim is further substantiated by studies showing that CB_1 receptors are located mainly on pre-synaptic terminals of GABAergic cells in the substantia nigra pars reticulata (Chan et al., 1998; Wallmichrath and Szabo, 2002), hippocampus (Wilson and Nicoll, 2001), amygdala (Katona et al., 2001) and in the VTA (Szabo et al., 2002) where they exert inhibitory effects on transmitter release. This indirect inhibition of GABA afferents may explain the excitatory profile observed in VTA neurons observed in the present study.

The results presented here indicate that HU210 exerted its effects via CB_1 receptors, a finding that has already been observed *in vitro* and *in vivo* (French, 1997; Cheer et al., 2000b, respectively). Pre-treatment with the cannabinoid receptor antagonist SR completely

prevented HU210-evoked effects on all simultaneously recorded neurons but was without effect on baseline firing activity. An interesting finding was that addition of HU210 in the presence of SR tended to decrease the firing rate of recorded neurons, an observation made by French (1997) when the antagonist was injected alone. This decrease in activity confirms the complex pharmacology of this compound which has been shown to act as an inverse agonist (Landsman et al., 1997). However, other researchers have found no effects of SR on basal firing rate of DA neurons, arguing against a modulatory tone of these cells by endogenous cannabinoids (Gueudet et al., 1995).

In order to dissect the pharmacological profile of simultaneously recorded neurons, rats were injected with the D₂ receptor agonist apomorphine when the change in firing rate brought about by HU210 had stabilized. Apomorphine was utilized due to the exquisite sensitivity of midbrain dopaminergic neurons to this agent (Gueudet et al., 1995). The finding that a majority of neurons were susceptible to the inhibitory effects of apomorphine (70%; Hyland et al., 2002) supports the contention that the majority of cells whose activity was recorded were dopaminergic. However, in a report by Cameron et al. (1997), the authors found a group of neurons that hyperpolarize to DA but failed to show tyrosine hydroxylase (TH) immunoreactivity express D₂ receptors, challenging the view that only TH positive (dopaminergic) cells contain these receptors. In the present study, the majority of neurons that were excited by HU210 and inhibited by apomorphine had low basal firing rates (~4 Hz) and bursting profiles typical of dopaminergic neurons in the midbrain (Wu and French, 2000). Conversely, neurons that were inhibited by the cannabinoid agonist and by apomorphine had consistently higher firing rates (~9 Hz) and did not exhibit burst firing, suggesting that these neurons may have been GABAergic projection neurons (Steffensen et al., 1998). Previous anatomical studies have shown that VTA neurons are negative for TH staining suggesting that they lie in close proximity to TH-positive DA neurons which are thought to be GABAergic neurons (Oertel and Mugniani, 1984). Neurons that showed reciprocal responses were recorded from wires spatially separated by no more than 100 microns and it is known that dendrites of midbrain DA cells can release DA that activates D₂ auto- and heteroreceptors leading to inhibition of their firing (Missale et al., 1998). It is likely that when one recorded cell was increasing its firing due to inhibition of GABA release another cell in proximity was being inhibited by DA released from the excited cell.

The novel and unexpected pharmacological effects seen in this chloral hydrate anesthetized VTA preparation; namely cannabinoid-induced inhibitions, provides useful hints for subtle differences between recorded neurons. The present results would argue for

different neuronal populations responding in a heterogeneous manner to the treatments. In addition to the effects of cannabinoids on DA and GABA transmission, these compounds are also capable of modulating the release of a variety of other neurotransmitters (see Schlicker and Kathmann, 2001, for a comprehensive review). For instance, cannabinoids are known to decrease the release of glutamate in the hippocampus and the cerebellum (Shen et al., 1996; Takahashi and Linden, 2000). The inhibitory responses seen in our study could have been caused by a cannabinoid receptor-mediated decrease in glutamate release from PFC pyramidal neurons which project exclusively to DA neurons that project back to the PFC (Carr and Sesack, 2000). The small number of cells inhibited in our study is in agreement with estimates suggesting that GABA neurons comprise 15–20% of the total neuronal population in the VTA (Oertel and Mugniani, 1984). Moreover, direct postsynaptic effects of cannabinoids have been reported on several membrane conductances (Mackie et al., 1995; Schweitzer, 2000). Thus, it is likely that HU210 could have exerted inhibitory effects through an as yet unidentified post-synaptic CB₁ receptor coupled to hyperpolarizing potassium currents on VTA DA cells (Di Marzo et al., 1998). The recent findings that cannabinoids can directly inhibit the firing rate of cerebellar interneurons via the activation of a small potassium conductance supports this hypothesis (Kreitzer et al., 2002). The lack of effect of cannabinoids in a minority of neurons observed in this study is more difficult to interpret since it is unlikely that some neurons in the VTA are simply not affected by the complex synaptic actions of cannabinoids. Rather, it is possible that cells under opposing synaptic influences at the time recordings were carried out may have been temporarily rendered unresponsive to the cannabinoid challenge.

In summary, the present multiple single-unit study shows that cannabinoids are capable of eliciting differential neuronal firing in the VTA via activation of central cannabinoid receptors. Since VTA mesolimbic pathways have been found to play a role in the reinforcing properties of drugs of abuse, the present study lends support to the notion that both DA-dependent and DA-independent components interact to form a final common pathway in processes mediating cannabinoid-induced effects in reinforcement-related brain pathways.

Acknowledgements

The expert technical assistance of Clare Roe is greatly appreciated. We also thank Dr Yolanda Mateo for helpful suggestions regarding the manuscript.

References

- Arevalo, C., de Miguel, R., Hernandez-Tristan, R., 2001. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol. Biochem. Behav* 70 (1), 123–131.
- Braida, D., Pozzi, M., Cavallini, R., Sala, M., 2001a. Conditioned place preference induced by the cannabinoid agonist CP55,940: Interaction with the opioid system. *Neuroscience* 104 (4), 923–926.
- Braida, D., Pozzi, M., Cavallini, R., Sala, M., 2001b. Intracerebral self-administration of the cannabinoid receptor agonist CP55,940 in the rat: interaction with the opioid system. *Eur. J. Pharmacol* 413, 227–234.
- Cameron, D.L., Wessendorf, M.W., Williams, J.T., 1997. A subset of ventral tegmental area neurons is inhibited by dopamine, 5-hydroxytryptamine and opioids. *Neuroscience* 77 (1), 155–166.
- Carr, D.B., Sesack, S.R., 2000. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J. Neurosci.* 20 (10), 3864–3873.
- Chan, P., Chan, S., Yung, W., 1998. Presynaptic inhibition of GABAergic inputs to rat substantia nigra pars reticulata neurones by a cannabinoid agonist. *Neuroreport* 9, 671–675.
- Cheer, J.F., Kendall, D.A., Marsden, C.A., 2000a. Cannabinoid receptors and reward in the rat: a conditioned place preference study. *Psychopharmacology (Berl)* 151, 25–30.
- Cheer, J.F., Marsden, C.A., Kendall, D.A., Mason, R., 2000b. Lack of response suppression follows repeated ventral tegmental cannabinoid administration: an in vitro electrophysiological study. *Neuroscience* 99 (4), 661–667.
- Chen, J.P., Marmur, R., Pulles, A., Paredes, W., Gardner, E.L., 1993. Ventral tegmental microinjection of delta 9-tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient. *Brain Res* 621 (1), 65–70.
- Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci* 21 (12), 521–528.
- Fattore, L., Cossu, G., Martellotta, C.M., Fratta, W., 2001. Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. *Psychopharmacology (Berl)* 156 (4), 410–416.
- Floresco, S.B., Todd, C.L., Grace, A.A., 2001. Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *J. Neurosci.* 21 (13), 4915–4922.
- French, E.D., 1997. D9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci. Letts* 226, 159–162.
- Gardner, E.L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., Morrison, D., 1988. Facilitation of brain stimulation reward by delta-9-tetrahydrocannabinol. *Psychopharmacology (Berl.)* 96, 142–144.
- Gueudet, C., Santucci, V., Rinaldi-Carmona, M., Soubrie, P., Le Fur, G., 1995. The CB₁ receptor antagonist SR141716A affects A₉ dopamine neuronal activity in the rat. *Neuroreport* 6, 1293–1297.
- Hernandez, M., Berrendero, F., Suarez, I., Garcia-Gil, L., Cebeira, M., Mackie, K., Ramos, J.A., Fernandez-Ruiz, J., 2000. Cannabinoid CB(1) receptors colocalize with tyrosine hydroxylase in cultured fetal mesencephalic neurons and their activation increases the levels of this enzyme. *Brain Res* 857 (1-2), 56–65.
- Hökfelt, T., Skirboll, L., Rehfeld, J.F., Goldstein, M., Markey, K., Dann, O., 1980. A sub-population of mesencephalic dopamine neurons projecting to limbic areas contain a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. *Neuroscience* 5, 2093–2124.
- Hyland, B., Reynolds, J., Hay, J., Perk, C., Miller, R., 2002. Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114 (2), 475.
- Katona, I., Rancz, E.A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., Freund, T.F., 2001. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21 (23), 9506–9518.
- Kreitzer, A.C., Carter, A.G., Regehr, W.G., 2002. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron* 34 (5), 787–796.
- Landsman, R.S., Burkey, T.H., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. SR141716A is an inverse agonist at the human cannabinoid receptor. *Eur. J. Pharmacol.* 334, 1–2.
- Mackie, K., Lai, Y., Westenbroek, R., Mitchell, R., 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J. Neurosci.* 15 (10), 6552–6561.
- Mansbach, R.S., Nicholson, K.L., Martin, B.R., Balster, R.L., 1994. Failure of Delta(9)-tetrahydrocannabinol and CP 55,940 to maintain intravenous self-administration under a fixed-interval schedule in rhesus monkeys. *Behav. Pharmacol* 5 (2), 219–225.
- Martelotta, M.C., Cossu, G., Fattore, L., Gessa, G.L., Fratta, W., 1998. Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naïve mice. *Neuroscience* 85, 327–330.
- Mechoulam, R., 1986. The pharmacology of cannabis sativa. In: Mechoulam, R. (Ed.), *Cannabinoids as Therapeutic Agents*. CRC Press, Boca Raton, FL, pp. 1–16.
- Melis, M., Gessa, G.L., Diana, M., 2000. Different mechanisms for dopaminergic excitation induced by opiates and cannabinoids in the rat midbrain. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 24 (6), 993–1000.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. *Physiol. Rev* 78 (1), 189–225.
- Oertel, W.H., Mugnani, E., 1984. Immunocytochemical studies of GABAergic neurons in rat basal ganglia and their relations to other neuronal systems. *Neurosci. Lett* 47 (3), 233–238.
- Paxinos, G., Watson, C., 1986. *The Rat Brain in Stereotaxic Coordinates*, 2nd ed. Academic Press, Sydney, Australia.
- Schlicker, E., Kathmann, M., 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci* 22 (11), 565–572.
- Schweitzer, P., 2000. Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. *J. Neurosci.* 20 (1), 51–58.
- Self, D.W., Nestler, E.J., 1995. Molecular mechanisms of drug reinforcement and addiction. *Annu. Rev. Neurosci* 18, 463–495.
- Shen, M., Piser, T.M., Seybold, V.S., Thayer, S.A., 1996. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J. Neurosci.* 16 (14), 4322–4334.
- Steffensen, S.C., Svingos, A.L., Pickel, V.M., Henriksen, S.J., 1998. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J. Neurosci.* 18 (19), 8003–8015.
- Szabo, B., Siemes, S., Wallmichrath, I., 2002. Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. *Eur. J. Neurosci.* 15 (12), 2057–2061.
- Takahashi, K.A., Linden, D.J., 2000. Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J. Neurophysiol* 83 (3), 1167–1180.
- Tanda, G., Pontieri, F.E., Di Chiara, G., 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common opioid receptor mechanism. *Science* 276, 2050–2054.
- Tanda, G., Munzar, P., Goldberg, S.R., 2000. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nat. Neurosci* 3 (11), 1073–1074.
- Tzschentke, T.M., 2001. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog. Neurobiol* 63 (3), 241–320.
- Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 410 (6828), 588–592.

Wallmichrath, I., Szabo, B., 2002. Cannabinoids inhibit striatonigral GABAergic neurotransmission in the mouse. *Neuroscience* 113 (3), 671–682.

Wu, X., French, E.D., 2000. Effects of chronic Δ^9 -tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. *Neuropharmacology* 3, 391–398.