Cannabinoid Receptor Localization in Brain: Relationship to Motor and Reward Systems

MILES HERKENHAM

Section on Functional Neuroanatomy
National Institute of Mental Health
Building 36, Room 2D-15
Bethesda, Maryland 20892

INTRODUCTION

Marijuana (*Cannabis sativa*) is one of the oldest and most widely used drugs in the world, with a history of use dating back over 4,000 years.[1,2] It was not until about twenty years ago that the principal psychoactive ingredient of the marijuana plant was isolated and found to be Δ^9-tetrahydrocannabinol (Δ^9-THC).[3-5] Δ^9-THC and other natural and synthetic cannabinoids produce characteristic behavioral and cognitive effects,[6,7] most of which can be attributed to actions on the central nervous system.[8] Marijuana use in this country is widespread, with approximately 40 million Americans having at least tried the drug.[9] Compulsive use is associated with social and psychological problems in individuals.[10] There is little evidence of adverse health side effects or toxicity.[7,11-13]

Until recently, very little was known about the cellular mechanisms through which cannabinoids act. The unique spectrum of cannabinoid effects and the stereoselectivity (enantioselectivity) of action of cannabinoid isomers in behavioral studies (see below) strongly suggested the existence of a specific cannabinoid receptor in brain, but early attempts to identify and characterize such a recognition site were not successful (discussed in refs. 14–16).

Without evidence that cannabinoids act through a specific receptor coupled to a functional effector system, researchers were prone to study the effects of cannabinoids on membrane properties, membrane-bound enzymes, eicosanoid production, metabolism, and other neurotransmitter systems *in vitro*.[8,17-19] As pointed out before,[20] most of the biochemical studies employed concentrations of Δ^9-THC that were in excess of physiologically meaningful concentrations that might be found in brain (for review, see refs. 8, 18). In addition, the criterion of structure-activity relationship was not met—that is, the potencies of various cannabinoids in the *in vitro* assays did not correlate with their relative potencies in eliciting characteristic behavioral effects.[8,20] Particularly damaging to the relevance of these *in vitro* studies was the absence of enantioselectivity.[20]

However, several groups have reported enantioselectivity of THC isomers in various behavioral tests *in vivo*. Martin's group found that the potencies of (−) and (+) forms of each of the *cis* and *trans* isomers of Δ^9-THC differ by 10- to 100-fold in producing

static ataxia in dogs, depressing schedule-controlled responding in monkeys, and in producing hypothermia and inhibiting spontaneous activity in mice.[21] Hollister *et al.*[22] showed cannabinoid enantioselectivity in human studies using indices of the subjective experience, or "high." May's group found enantioselectivity of a series of synthetic cannabinoids in tests of motor depression and analgesia.[23-25]

One of May's compounds, (−)-9-nor-9β-hydroxyhexahydrocannabinol (β-HHC), was used as a lead compound by Johnson and Melvin[26] for the synthesis of a rather large series of structurally novel, classical and nonclassical, cannabinoids for studies of their potential use as analgesics (FIG. 1). The synthetic cannabinoids share physicochemical properties with the natural cannabinoids and produce many behavioral and physiological effects characteristic of Δ^9-THC, but are 5–1000 times more potent and show high enantioselectivity.
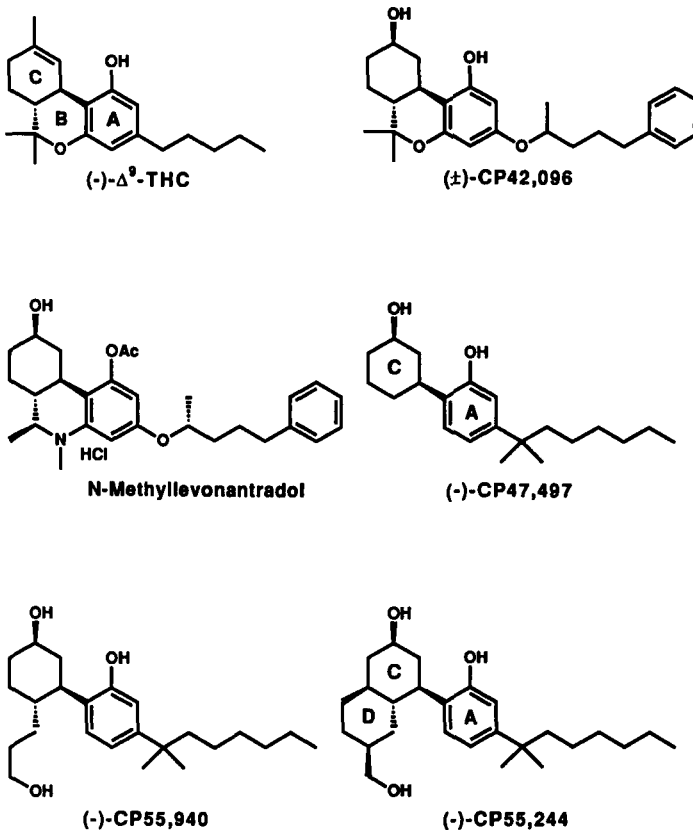
FIGURE 1. Chemical structures of Δ^9-tetrahydrocannabinol (Δ^9-THC) and three synthetic cannabinoids. According to the nomenclature of Johnson and Melvin,[26] Δ^9-THC and 9-Nor-9β-hydroxyhexahydrocannabinol (β-HHC) are defined as members of the ABC-tricyclic cannabinoid class. CP 55,940 is a hydroxypropyl analog of a 2-(3-hydroxycyclohexyl)phenol, defined as an AC-bicyclic cannabinoid. CP 55,244 is an ACD-tricyclic cannabinoid with a rigidly positioned hydroxypropyl moiety. (Reproduced from Herkenham *et al.*[16])

The availability of the nonclassical compounds revolutionized the study of the biochemical basis of cannabinoid activity. Howlett's group used them in neuroblastoma cell lines to show inhibition of adenylate cyclase activity.[27] Such inhibition is enantioselective, and the pharmacological profile correlates well with that observed by Martin's group in tests of mouse spontaneous activity, catalepsy, body temperature, and analgesia.[28]

One of the nonclassical compounds, CP 55,940, was tritiated and used by Howlett's group to identify and fully characterize a unique cannabinoid receptor in membranes from rat brain.[14] The results from the centrifugation assay showed that [3H]CP 55,940 receptor binding is saturable, has high affinity and enantioselectivity, and exhibits characteristics expected for a neuromodulator receptor associated with a guanine nucleotide regulatory (G) protein.

Recently, we characterized and validated the binding of [3H]CP 55,940 in slide-mounted brain sections and described assay conditions to autoradiographically visualize the CNS distribution of cannabinoid receptors in a number of mammals, including humans.[29] Autoradiography revealed a unique distribution that is similar in all mammalian species examined; binding is most dense in outflow nuclei of the basal ganglia—the substantia nigra pars reticulata and globus pallidus—and in the hippocampus and cerebellum.

The localization of dense receptors in the outflow nuclei of the basal ganglia may account for some of the actions of cannabinoids. Dense binding localized in the globus pallidus, entopeduncular nucleus, and substantia nigra pars reticulata suggests an association of cannabinoid receptors with striatal efferent projections to these nuclei and, therefore, a role for cannabinoids in motor control. In addition, binding may be localized on mesostriatal dopaminergic neurons, which would implicate a role for cannabinoids in direct control of dopamine release and, therefore, brain reward mechanisms.

This report summarizes several key features of our cannabinoid receptor localization studies: 1) validation that the *in vitro* binding in brain sections is the same binding that mediates the effects of cannabinoids *in vivo*; 2) general features of brain distribution in several species, including human; and 3) neuronal localization of cannabinoid receptors to motor and/or limbic components of the basal ganglia, assessed by making selective chemical lesions of either the striatal GABAergic efferent or dopaminergic afferent pathways interconnecting the caudate-putamen (CPu) and the substantia nigra.

MATERIALS AND METHODS

Binding Assays

Cannabinoid Receptor Binding

The procedures for obtaining cryostat-cut sections of fresh, frozen brain mounted on "subbed" microscope slides were described previously.[15,29] Assay conditions yielding 80–90% specific binding are: incubation of slide-mounted sections at 37°C for 2 h in 50 mM Tris-HCl buffer, pH 7.4, with 5% bovine serum albumin (BSA) and 1–10 nM [3H]CP 55,940 (sp. act. 76 Ci/mmole). Slides are washed at 0°C for 4 h in the same buffer with 1% BSA and then dried. For use in competition studies to characterize and validate binding, natural and synthetic cannabinoid ligands were obtained from the

National Institute of Drug Abuse and Pfizer, Inc. Names and stereochemical configurations of some of the cannabinoids are shown in FIGURE 1.

Autoradiography was performed on 15–25 μm-thick brain sections of rat (Sprague-Dawley), guinea pig (Hartley), dog (beagle), rhesus monkey and human (dying of non-neurological disorders). Sections were incubated in 10 nM [^{3}H]CP 55,940 using optimized conditions, then washed, dried, and exposed to tritium-sensitive film (LKB or Amersham) for 3 to 4 weeks before developing. Developed films were digitized with a solid-state video camera and Macintosh II computer-based system for densitometry. Receptor densities were quantified using IMAGE® software (Wayne Rasband, Research Services Branch, NIMH).

Dopamine D_1 and D_2 Receptor Binding

Both the D_1 and D_2 receptor assays were carried out as previously described.[30–32] For D_1 receptor binding, slides were warmed to room temperature and incubated at 25°C for 2.5 h in 25 mM Tris-HCl buffer, pH 7.5, with 100 mM NaCl, 1 mM MgCl$_2$, 0.001% ascorbate, and 0.55 nM [^{3}H]SCH 23390 (sp. act. 74.8 Ci/mmole). Slides were washed for 10 min in the same buffer at 4°C, dipped in deionized water, and dried. Nonspecific binding was determined by addition of 2 μM cis-flupenthixol and was typically <5% of total binding. Sections were exposed to film for 2 weeks.

For D_2 receptor binding, sections were incubated at 25°C for 1.5 h in 25 mM Tris-HCl buffer, pH 7.5, with 200 mM NaCl, 1 mM MgCl$_2$, 0.001% ascorbate, and 1.5 nM [^{3}H]raclopride (sp. act. 64 Ci/mmole). They were washed for 3 min in the same buffer at 4°C with 100 mM NaCl. Nonspecific binding, determined by addition of 5 μM sulpiride, was typically <5% of total binding. Sections were exposed to film for 3 weeks.

Dopamine Uptake Site

Assay conditions were described previously.[33,34] Slides were incubated at 2°C for 30 h in 50 mM sodium phosphate buffer, pH 7.5, with 120 mM NaCl, 0.01% BSA, 0.001% ascorbate, 500 nM trans-flupenthixol, and 0.25 nM [^{3}H]GBR 12935 (sp. act. 53.1 Ci/mmole). They were washed for 2 h in same buffer at 2°C. Nonspecific binding, determined by addition of 20 μM mazindol, was typically <15% of total binding. Sections were exposed to film for 8 weeks.

Lesions

Striatal Ibotenate Lesions

Male rats (Sprague Dawley) were anesthetized and placed in a stereotaxic frame. A cannula was lowered into the caudate-putamen. Via an infusion pump and tubing, 1.5 μl (7.5 μg) of ibotenate dissolved in normal saline was infused over 8 min. Animals survived for 2 or 4 weeks before sacrifice by decapitation.

6-OHDA Lesions

Rats were prepared as above but were injected i.p. with desmethylimipramine (15 mg/kg) 30 min before infusion. The cannula was lowered into the medial forebrain bundle (mfb). Four μl (8 μg) of 6-OHDA dissolved in normal saline with 0.1% ascorbate added was infused over 8 min. Animals survived for 4 weeks before sacrifice; at 2 weeks post-lesion they were tested for rotational behavior 40 min after administration of 5 mg/kg of d-amphetamine sulfate (Sigma). Only those rats showing greater than 10 rotations per min during a 5-min test were used in the binding experiment.

RESULTS

A large series of cannabinoid and non-cannabinoid drugs was assayed to test for competitive displacement of [³H]CP 55,940 (TABLE 1). The competition curves and derived inhibition constants (K_i's) for the natural and synthetic cannabinoids provided a test for validation of binding. We found that highly significant ($p < 0.0001$) correlations exist between the K_i's and potencies of the drugs in tests of dog ataxia and human subjective experience, the two most reliable markers of cannabinoid activity.[6,7] The K_i's also correlate very closely with relative potencies in tests of motor function (ataxia, hypokinesia, catalepsy), analgesia, and inhibition of contractions of guinea pig ileum and adenylate cyclase in neuroblastoma cell lines in vitro.[29] Enantioselectivity is striking; the (–) and (+) forms of CP 55,244 differ by more than 10,000-fold in vitro, a separation predicted by the rigid structure of the molecule (FIG. 1)[26] and by potencies in vivo. Natural cannabinoids lacking psychoactive properties, such as cannabidiol, show extremely low potency at the receptor, and all tested non-cannabinoid drugs have no potency (TABLE 1).

Autoradiography showed that in all species very dense binding is found in the globus pallidus, substantia nigra pars reticulata (SNR), and the molecular layers of the cerebellum and hippocampal dentate gyrus (FIGS. 2 and 3). Dense binding is also found in the cerebral cortex, other parts of the hippocampal formation, and striatum. In rat, rhesus monkey and human, the SNR contains the highest level of binding (FIG. 3). In dog, the cerebellar molecular layer is most dense (FIG. 2H). In guinea pig and dog, the hippocampal formation has selectively dense binding (FIG. 2E, F). Neocortex in all species has moderate binding across fields, with peaks in superficial and deep layers. Very low and homogeneous binding characterizes the thalamus and most of the brainstem, including all of the monoamine-containing cell groups, reticular formation, primary sensory, visceromotor and cranial motor nuclei, and the area postrema. The exceptions—hypothalamus, basal amygdala, central gray, nucleus of the solitary tract, and laminae I–III and X of the spinal cord—show slightly higher but still sparse binding (FIGS. 2 and 3).

Quantitative autoradiography reveals very high numbers of receptors, exceeding 1 pmole/mg protein in densely labeled areas. Thus, cannabinoid receptor density is far in excess of densities of neuropeptide receptors and is similar to levels of cortical benzodiazepine,[35] striatal dopamine,[30,36] and whole-brain glutamate receptors.[37]

TABLE 1. Potencies of Some Cannabinoids in the Section Binding and Other Assays

| Compound	Ki (nM)	Catalepsy/ Ataxia (mg/kg)	Mouse Analgesia (mg/kg)	Cyclase Inhibition (nM)	Human "High" (mg)
CP 55,940 (−AC)	15 (Kd)	0.35	0.7	25	
CP 56,667 (+AC)	470	>10	>15	>5,000	
CP 55,244 (−ACD)	1.4	0.09	0.09	5	
CP 55,243 (+ACD)	18000	>10	>10	>10,000	
CP 50,556	14	1.5	0.4	100	0.5
CP 53,870	26000	>10	6.5	>5,000	
CP 54,939	14	0.05	0.7	7	
Nabilone	120	0.03		100	1
β-HHC	124	0.1	1.6		
α-HHC	2590	0.5	>50		
(−)Δ^9-THC	420	0.5	0.5	10	1
(+)Δ^9-THC	7700	>2.0	>100		
Δ^8-THC	498	0.5	8.8		2
11-OH-Δ^9-THC	210	0.05	1.9		1
TMA-Δ^8-THC	2300				
8β-OH-Δ^9-THC	4200				10
8α-OH-Δ^9-THC	8700				10
11-OH-Cannabinol	800				
Cannabinol	3200				>15
Cannabidiol	53000	inactive	>100		>30
Cannabigerol	275000	>7			
9-COOH-11-nor-Δ^9-THC	75000	>40	10		
9-COOH-11-nor-Δ^8-THC	Inactive				

CP analogs were synthesized at Pfizer Central Research; their structures are given in Johnson and Melvin.[26] The first 6 analogs are enantiomeric pairs, as are α- and β-HHC and (−) and (+)Δ^9-THC. CP 50,556 is levonantradol; CP 53,870 is dextronantradol; CP 54,939 is desacetyl levonantradol; TMA-Δ^8-THC is trimethylammonium-Δ^8-THC. The last two compounds are Δ^9-THC metabolites. Ki's ± standard deviations are derived from binding surface analysis. Drugs which at 10 μM concentration show no inhibition of [³H]CP 55,940 binding are: amphetamine, β-estradiol, cis-flupenthixol (dopamine receptor ligand), cocaine, corticosterone, cyclohexyladenosine, dexamethasone, etorphine (opiate receptor ligand), γ-amino butyric acid (GABA), glutamate, leukotriene B₄ and D₄ (both at 1 μM), lysergic acid diethylamide (LSD), phencyclidine (PCP), prostaglandin E₂, Ro15-1788 (benzodiazepine receptor ligand), and thujone (the active ingredient of absinthe). (Modified from Herkenham et al.[29])

Striatal Ibotenate Lesions

The single injection of ibotenate into the caudate-putamen resulted in a small central site of nonspecific destruction marked by gliosis and a much larger (approximately 3 × 4 mm) surrounding area of selective neuronal degeneration, in accordance with previous descriptions of toxicity in the dose range and location used.[38] In the affected striatal territory, the losses on the lesion relative to corresponding territory on the control side were the most profound for the dopamine D₁ receptors, showing a 96% reduction in binding at 4 weeks (TABLE 2). Cannabinoid and D₂ receptors were reduced
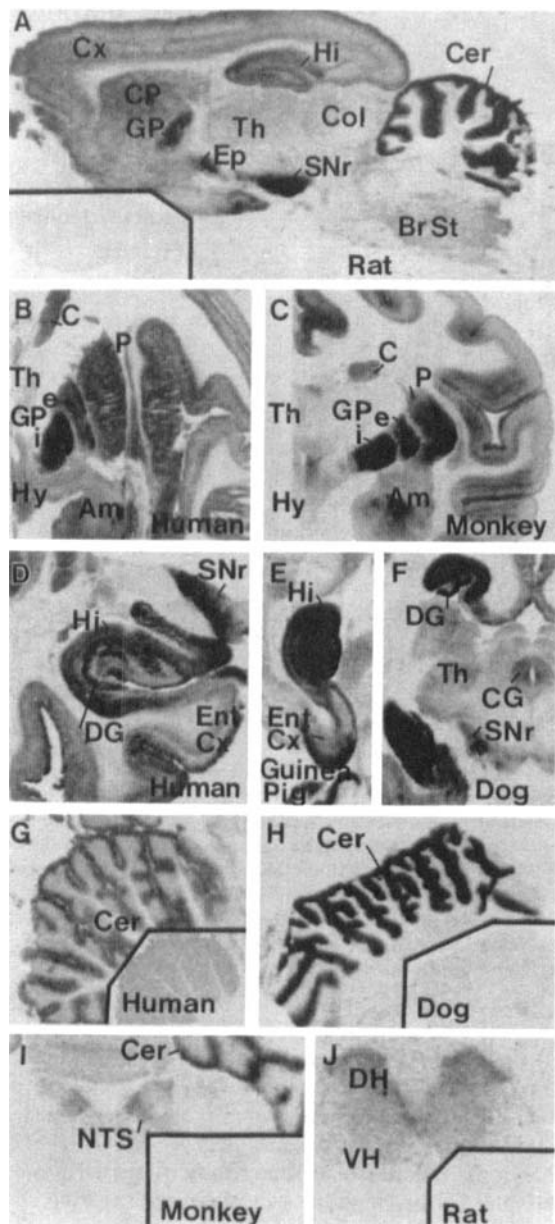
FIGURE 2. Autoradiography of 10 nM [³H]CP 55,940 binding in brain. Tritium-sensitive film exposed for 4 weeks, developed and computer digitized. Images were photographed directly from the computer monitor. Gray levels represent relative levels of receptor densities. Sagittal section of rat brain in **A**. Coronal brain sections of human in **B**, **D**, and **G**; rhesus monkey in **C** and **I**; dog in **F** and **H**; and rat in **J**. Horizontal section of guinea pig brain in **E**. *Insets* show non-specific binding in adjacent sections (miniaturized images are shown). *Abbreviations:* Am, amygdala; Br St, brain stem; Cer, cerebellum; CG, central gray; C, caudate; Col, colliculi; CP, caudate-putamen; Cx, cerebral cortex; DG, dentate gyrus; DH, dorsal horn of spinal cord; Ent Cx, entorhinal cortex; Ep, entopeduncular nucleus (homolog of GPi); GP, globus pallidus (e, external; i, internal); Hi, hippocampus; Hy, hypothalamus; NTS, nucleus of solitary tract; P, putamen; SNr, substantia nigra pars reticulata; Th, thalamus; VH, ventral horn of spinal cord. (Reproduced from Herkenham *et al.*[29])
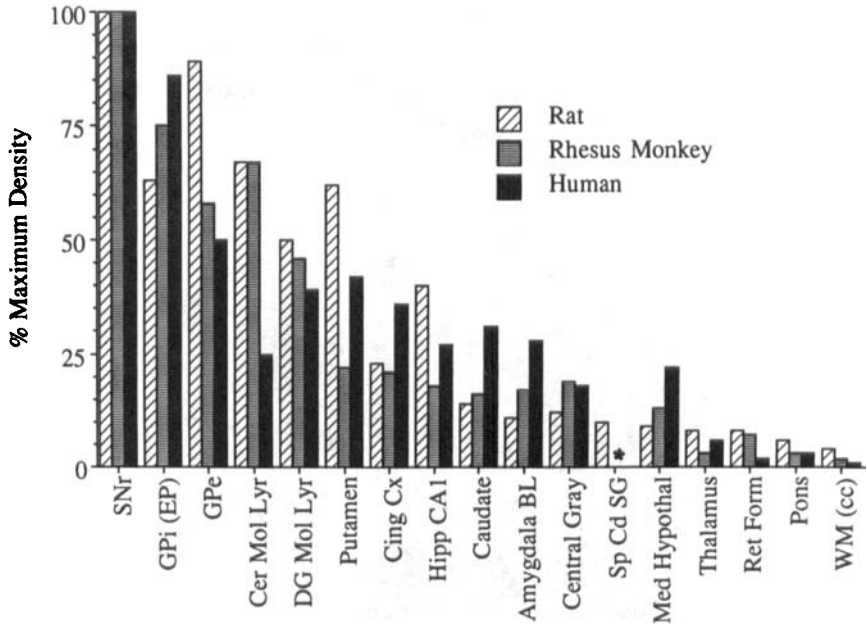
FIGURE 3. Relative densities of cannabinoid receptors across brain structures in rat, rhesus monkey, and human. Autoradiographic images were digitized by a solid-state camera and Macintosh II computer-based system for quantitative densitometry using Image⊖ software (Wayne Rasband, Research Services Branch, NIMH). Transmittance levels were converted to fmoles/mg tissue using tritium standards, then normalized to the densest structure in each animal (SNr for all three). For every section incubated for total binding, an adjacent section was incubated in the presence of CP 55,244 to permit subtraction of nonspecific binding on a regional basis. Structure abbreviations not given in FIGURE 2 legend are: Cing Cx, cingulate cortex; Hipp CA1, hippocampal field CA1; Med Hypothal, medial hypothalamus; Sp Cd SG, substantia gelatinosa of spinal cord (*only rat measured); Ret Form, reticular formation; WM (cc), white matter of corpus callosum. (Reproduced from Herkenham *et al.*[29])

by 78–80% in the affected territory, and the dopamine uptake site, which resides on afferent dopaminergic axons, was slightly reduced at 4 weeks (not shown).

Both cannabinoid and D_1 dopamine receptors are lost in similar patterns (FIG. 4) and amounts (TABLE 2) in projection zones of lesioned striatal efferent neurons. In agreement with known medial-lateral topography of striatal projections,[39] receptor losses in both GP and SNR were greatest medially, with sparing of binding in lateral parts receiving projections from unlesioned parts of the lateral and posterior caudate-putamen (FIG. 4). For cannabinoid receptor binding, losses in the labeled striatonigral pathway were also evident (not shown).

Mfb 6-OHDA Lesions

Nissl-stained sections of the substantia nigra showed unilateral loss of neurons in the SNC (FIG. 4e). Autoradiography showed no change in cannabinoid receptor

TABLE 2. Effects of Unilateral Striatal Ibotenate Lesions at 4 Week Survival ($n = 5$)

Structure	Cannabinoid Receptor	L % R	D-1 Receptor	L % R	D-2 Receptor	L % R
CPu (L)	536 ± 37	20	69 ± 18	4	54 ± 25	18
(R)	2843 ± 849		1558 ± 111		299 ± 22	
GP (L)	719 ± 182	15	13 ± 6	12		
(R)	4763 ± 969		106 ± 28			
EP (L)	905 ± 340	22	57 ± 23	24		
(R)	4159 ± 834		240 ± 39			
SNR (L)	1023 ± 174	16	164 ± 16	13		
(R)	6421 ± 635		1254 ± 97			

Values are in fmoles/mg protein and are the means and standard deviations of specific binding to approximately 50% of the total number (B_{max}) of receptors and 10% of uptake sites in each region, since ligand concentrations were near the K_d or below, in the case of [^{3}H]GBR-12935, for each drug. Corresponding locations on the control (R) side were outlined and measured. All left–right differences are significant at the $p < 0.005$ level of confidence except for the dopamine uptake site, which is significant at the $p < 0.05$ level (Student's paired t-test). Abbreviations: CPu, caudate putamen; EP, entopeduncular nucleus; GP, globus pallidus; SNR, substantia nigra pars compacta. (Data are from Herkenham et al.[32])

binding in either the striatum (FIG. 4d) or the nigra (FIG. 4e), whereas dopamine uptake sites were lost throughout the striatum and nigra on the lesioned side (FIG. 4f). Quantitative densitometry showed major losses of dopamine uptake sites in the caudate-putamen, accumbens nucleus (ACb), and substantia nigra pars compacta (SNC), but no loss of cannabinoid receptors (TABLE 3).

DISCUSSION

The section binding assay is easy to perform, is reliable, and shows high sensitivity to manipulations of binding conditions, such as the addition of guanine nucleo-

TABLE 3. Effect of Unilateral 6-OHDA Lesions at 4 Week Survival ($n = 4$)

Structure	Cannabinoid Receptor	L % R	Dopamine Uptake Site	L % R
CPu (L)	4395 ± 266	99	62 ± 57	20
(R)	4422 ± 282		311 ± 60	
ACb (L)	2533 ± 384	102	38 ± 30	39
(R)	2491 ± 463		98 ± 13	
SNC (L)	1709 ± 320	95	1 ± 1	7
(R)	1807 ± 264		17 ± 7	

Values are in fmoles/mg protein and are the means and standard deviations of specific binding to approximately 50% and 10% of the total number (B_{max}) of receptors and uptake sites in each region, respectively. Densitometric measures of caudate-putamen (CPu), nucleus accumbens (ACb), and substantia nigra pars compacta (SNC) were each taken from the entire structure at the levels shown in FIGURE 3. Left–right cannabinoid receptor differences were not significant (Student's paired t-test); dopamine uptake site left–right differences were significant in the CPu ($p < 0.01$), ACb ($p = 0.05$), and SNC ($p < 0.03$). (Data are from Herkenham et al.[32])
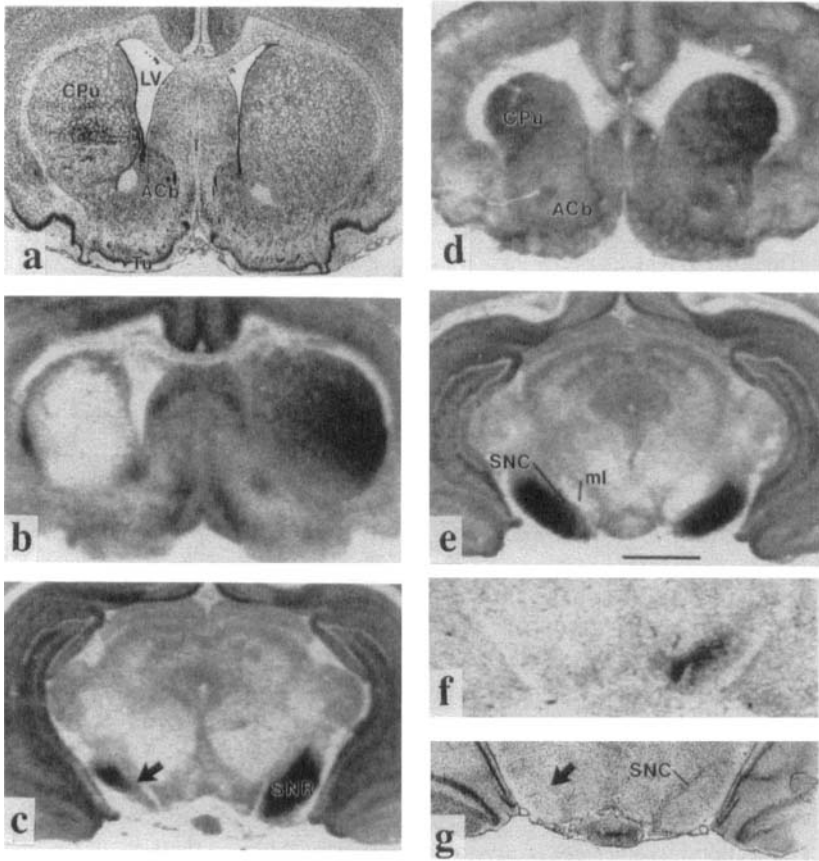
FIGURE 4. Lesion data showing localization of cannabinoid receptors to striatonigral neurons (a–c) and not to dopaminergic nigrostriatal neurons (d–g). As shown in a–c, a unilateral deposit of ibotenate was placed into the caudate-putamen. Nissl-stained section in a shows the area of selective neuronal loss and the enlarged lateral ventricle (LV). At 4 weeks survival, the losses of cannabinoid receptors in the caudate-putamen (b) and substantia nigra pars reticulata (SNR) (*arrow* in c) are shown autoradiographically. The losses are topographic; note sparing of laterally situated striatal neurons and their nigral projections. As shown in d–g, a unilateral lesion of the mesencephalic ascending dopamine system was made by depositing 6-OHDA into the medial forebrain bundle. At 4 weeks survival, degeneration of dopamine neurons in the substantia nigra pars compacta (SNC) is evident in the Nissl stain (*arrow* in g) and by the losses of dopamine uptake sites in the SNC (f), whereas cannabinoid receptor binding is unaffected in the striatum (d) and nigra (e). *Abbreviations*: ACb, nucleus accumbens; ml, medial lemniscus; Tu, olfactory tubercle. Magnification bar measures 2 mm. (Modified from Herkenham et al.[32])

tides.[29] BSA appears to act as a carrier to keep cannabinoids in solution without appreciably affecting binding kinetics. The low nonspecific binding and absence of binding in white matter indicates that the autoradiographic patterns are not affected by ligand lipophilia. The inclusion of BSA in the incubation medium may actually mimic the disposition of cannabinoids administered *in vivo*, as they would quickly complex with serum albumin or other carriers in the blood.

The structure-activity profile suggests that the receptor defined by the binding of [³H]CP 55,940 is the same receptor that mediates many of the behavioral and pharmacological effects of cannabinoids (TABLE 1), including the subjective experience termed the human "high." All other tested psychoactive drugs, neurotransmitters, steroids, and eicosanoids at 10 μM concentrations failed to bind to this receptor. There was no compelling evidence for receptor subtypes from that analysis.

Autoradiography of cannabinoid receptors reveals a heterogeneous distribution pattern that conforms to cytoarchitectural and functional subdivisions in the brain. The distribution is unique—no other pattern of receptors is similar—and it is similar across several mammalian species, including human, suggesting that cannabinoid receptors are phylogenetically stable and conserved in evolution. The distribution appears to be similar to the distribution of the mRNA probe hybridized to a rat brain cannabinoid receptor gene.[40]

The locations of cannabinoid receptors help to understand cannabinoid pharmacology. High densities in the hippocampus and cerebral neocortex implicate roles for cannabinoids in cognitive functions. High densities in axons and terminals of the GABAergic striatal neurons of the basal ganglia and of glutamatergic granule cells of the cerebellum suggest a modulatory role in movement systems. Sparse densities in lower brainstem areas controlling cardiovascular and respiratory functions may explain why high doses of Δ^9-THC are not lethal.

The results of the 6-OHDA lesions indicate that cannabinoid receptors do not reside on mesencephalic dopamine neurons projecting to either the caudate-putamen or the ACb. Systemically administered Δ^9-THC has been shown to elevate extracellular levels of dopamine in the caudate-putamen[41] and ACb.[42] The mechanism of action appears to be indirect, as the effects are attenuated by naloxone.[42] Nevertheless, it has been proposed that drugs which elevate dopamine levels in the striatum are those that are known to have abuse liability in humans.[43,44] In humans, cannabinoids can produce a feeling of euphoria as part of the subjective experience known as the marijuana "high," but dysphoria, dizziness, thought disturbances, and sleepiness are also reported.[6,7,26] Animals generally will not self-administer Δ^9-THC.[45,46] Cannabinoids did not lower the threshold for electrical self-stimulation in one study.[47] In another study they did,[48] but apparently both this phenomenon and the enhancement of basal dopamine efflux from the ACb by Δ^9-THC are strain-specific, occurring only in Lewis rats.[49] Thus, the effects of cannabinoids on dopamine circuits thought to be common mediators of reward are indirect and different from those of drugs such as cocaine and morphine which directly affect extracellular dopamine levels and produce craving and powerful drug-seeking behavior.

Accounts of cannabis use in humans stress the loosening of associations, fragmentation of thought, and confusion on attempting to remember recent occurrences.[7,50] These cognitive effects may be mediated by receptors in the cerebral cortex, especially the receptor-dense hippocampal cortex. The hippocampus "gates" information during memory consolidation and codes spatial and temporal relations among stimuli and responses.[51,52] Δ^9-THC causes memory "intrusions,"[53] impairs temporal aspects of performance,[54] and suppresses hippocampal electrical activity.[55]

The localization of cannabinoid receptors in motor areas suggests therapeutic applications. Cannabinoids exacerbate hypokinesia in Parkinson's disease but are beneficial for some forms of dystonia, tremor, and spasticity.[6,7,56–58] The association of cannabinoid receptors with GABAergic striatal projection neurons suggests roles for cannabinoids in control of movement, perhaps therapeutic roles in hyperkinesis and dystonia. Cannabinoids have been shown to be beneficial for some forms of dystonia,

tremor, and spasticity.[6,7,56-59] Lack of association of cannabinoid receptors with dopamine neurons indicates that cannabinoids do not directly affect dopamine release associated with reward and drug-seeking behavior. Further work may show the basis for the reported usefulness in controlling nausea and stimulating appetite in patients receiving chemotherapy for cancer or AIDS. Finally, the development of an antagonist could lead to additional therapeutic applications. The section binding assay can be used to screen the potencies of novel drugs and serve to identify cannabinoid receptor subtypes, which could lead to renewed interest in developing cannabinoid drugs without unwanted side effects.

REFERENCES

1. HARRIS, L. S., W. L. DEWEY & R. K. RAZDAN. 1977. Cannabis. Its chemistry, pharmacology, and toxicology. *In* Handbook of Experimental Pharmacology. W. R. Martin, Ed. 45/II: 371–429. Springer-Verlag. New York.
2. MECHOULAM, R. 1986. The pharmacohistory of *Cannabis sativa. In* Cannabinoids as Therapeutic Agents. R. Mechoulam, Ed.: 1–19. CRC Press. Boca Raton, FL.
3. GAONI, Y. & R. MECHOULAM. 1964. Isolation, structure, and partial synthesis of an active constituent of hashish. J. Am. Chem. Soc. **86:** 1646.
4. MECHOULAM, R., A. SHANI, H. EDERY & Y. GRUNFELD. 1970. Chemical basis of hashish activity. Science **169:** 611–612.
5. MECHOULAM, R. 1973. Cannabinoid chemistry. *In* Marihuana. Chemistry, Pharmacology, Metabolism, and Clinical Effects. R. Mechoulam, Ed.: 1–99. Academic Press. New York.
6. DEWEY, W. L. 1986. Cannabinoid pharmacology. Pharmacol. Rev. **38:** 151–178.
7. HOLLISTER, L. E. 1986. Health aspects of cannabis. Pharmacol. Rev. **38:** 1–20.
8. MARTIN, B. R. 1986. Cellular effects of cannabinoids. Pharmacol. Rev. **38:** 45–74.
9. KOZEL, N. J. & E. H. ADAMS. 1986. Epidemiology of drug abuse: An overview. Science **234:** 970–974.
10. AMERICAN PSYCHOLOGICAL ASSOCIATION. 1987. Position statement on psychoactive substance use and dependence: Update on marijuana and cocaine. Am. J. Psychiatry **144:** 698–702.
11. BERKOW, R. 1987. The Merck Manual of Diagnosis and Therapy. 15. Merck Co. Rahway, NJ.
12. HAYES, J. S., M. C. DREHER & J. K. NUGENT. 1988. Newborn outcomes with maternal marihuana use in Jamaican women. Pediatr. Nurs. **14:** 107–110.
13. DILL, J. A. & A. C. HOWLETT. 1988. Regulation of adenylate cyclase by chronic exposure to cannabimimetic drugs. J. Pharmacol. Exp. Ther. **244:** 1157–1163.
14. DEVANE, W. A., F. A. I. DYSARZ, M. R. JOHNSON, L. S. MELVIN & A. C. HOWLETT. 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. **34:** 605–613.
15. HERKENHAM, M. 1991. Characterization and localization of cannabinoid receptors in brain: An *in vitro* technique using slide-mounted tissue sections. NIDA Res. Monogr. **112:** 129–145.
16. HERKENHAM, M., A. B. LYNN, M. R. JOHNSON, L. S. MELVIN, B. R. DE COSTA & K. C. RICE. 1991. Characterization and localization of cannabinoid receptors in rat brain: A quantitative in vitro autoradiographic study. J. Neurosci. **11:** 563–583.
17. HILLIARD, C. J., R. A. HARRIS & A. S. BLOOM. 1985. Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: Fluorescent studies. J. Pharmacol. Exp. Ther. **232:** 579–588.
18. PERTWEE, R. G. 1988. The central neuropharmacology of psychotropic cannabinoids. Pharmacol. Ther. **36:** 189–261.
19. REICHMAN, M., W. NEN & L. E. HOKIN. 1988. Δ^9-Tetrahydrocannabinol increases arachadonic acid levels in guinea pig cerebral cortex slices. Mol. Pharmacol. **34:** 823–828.
20. HOWLETT, A. C., M. BIDAUT-RUSSELL, W. A. DEVANE, L. S. MELVIN, M. R. JOHNSON & M. HERKENHAM. 1990. The cannabinoid receptor: Biochemical, anatomical and behavioral characterization. Trends Neurosci. **13:** 420–423.

21. MARTIN, B. R., R. L. BALSTER, R. K. RAZDAN, L. S. HARRIS & W. L. DEWEY. 1981. Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. Life Sci. 29: 565–574.
22. HOLLISTER, L. E., H. K. GILLESPIE, R. MECHOULAM & M. SREBNIK. 1987. Human pharmacology of 1S and 1R enantiomers of delta-3-tetrahydrocannabinol. Psychopharmacology 92: 505–507.
23. WILSON, R. S. & E. L. MAY. 1975. Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. J. Med. Chem. 18: 700–703.
24. WILSON, R. S., E. L. MAY, B. R. MARTIN & W. L. DEWEY. 1976. 9-Nor-9-hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with the tetrahydrocannabinols. J. Med. Chem. 19: 1165–1167.
25. WILSON, R. S., E. L. MAY & W. L. DEWEY. 1979. Some 9-hydroxycannabinoid-like compounds. Synthesis and evaluation of analgesic and behavioral properties. J. Med. Chem. 22: 886–888.
26. JOHNSON, M. R. & L. S. MELVIN. 1986. The discovery of nonclassical cannabinoid analgetics. In Cannabinoids as Therapeutic Agents. R. Mechoulam, Ed.: 121–145. CRC Press. Boca Raton, FL.
27. HOWLETT, A. C., M. R. JOHNSON, L. S. MELVIN & G. M. MILNE. 1988. Nonclassical cannabinoid analgesics inhibit adenylate cyclase: Development of a cannabinoid receptor model. Mol. Pharmacol. 33: 297–302.
28. LITTLE, P. J., D. R. COMPTON, M. R. JOHNSON & B. R. MARTIN. 1988. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J. Pharmacol. Exp. Ther. 247: 1046–1051.
29. HERKENHAM, M., A. B. LYNN, M. D. LITTLE, M. R. JOHNSON, L. S. MELVIN, B. R. DE COSTA & K. C. RICE. 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87: 1932–1936.
30. RICHFIELD, E. K., A. B. YOUNG & J. B. PENNEY. 1986. Properties of D2 dopamine receptor autoradiography: High percentage of high-affinity agonist sites and increased nucleotide sensitivity in tissue sections. Brain Res. 383: 121–128.
31. RICHFIELD, E. K., A. B. YOUNG & J. B. PENNEY. 1987. Comparative distribution of dopamine D-1 and D-2 receptors in the basal ganglia of turtle, pigeon, rat, cat, and monkey. J. Comp. Neurol. 262: 446–463.
32. HERKENHAM, M., A. B. LYNN, B. DE COSTA & E. K. RICHFIELD. 1991. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. Brain Res. 547: 267–274.
33. RICHFIELD, E. K. & M. HERKENHAM. 1989. Quantitative autoradiography of the dopamine uptake complex in the central nervous system. Soc. Neurosci. Abstr. 15: 1230.
34. RICHFIELD, E. K. 1990. Quantitative autoradiography of the dopamine uptake complex in rat brain using [³H]GBR 12935: Binding characteristics. Brain Res. 540: 1–13.
35. ZEZULA, J., R. CORTÉS, A. PROBST & J. M. PALACIOS. 1988. Benzodiazepine receptor sites in the human brain: Autoradiographic mapping. Neuroscience 25: 771–795.
36. BOYSON, S. J., P. MCGONIGLE & P. B. MOLINOFF. 1986. Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. J. Neurosci. 6: 3177–3188.
37. GREENAMYRE, J. T., A. B. YOUNG & J. B. PENNEY. 1984. Quantitative autoradiographic distribution of L-[³H]glutamate binding sites in rat central nervous system. J. Neurosci. 4: 2133–2144.
38. KÖHLER, C. & R. SCHWARCZ. 1983. Comparison of ibotenate and kainate neurotoxicity in rat brain: A histological study. Neuroscience 8: 819–835.
39. ALTAR, C. A. & K. HAUSER. 1987. Topography of substantia nigra innervation by D1 receptor containing striatal neurons. Brain Res. 410: 1–11.
40. MATSUDA, L. A., S. J. LOLAIT, M. J. BROWNSTEIN, A. C. YOUNG & T. I. BONNER. 1990. Structure of a cannabinoid receptor: Functional expression of the cloned cDNA. Nature 346: 561–563.
41. NG CHEONG TON, J. M., G. A. GERHARDT, M. FRIEDEMANN, A. M. ETGEN, G. M. ROSE, N. S. SHARPLESS & E. L. GARDNER. 1988. The effects of Δ⁹-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: An in vivo electrochemical and in vivo microdialysis study. Brain Res. 451: 59–68.
42. CHEN, J., W. PAREDES, J. LI, D. SMITH, J. LOWINSON & E. L. GARDNER. 1990. Δ⁹-

Tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology 102: 156–162.

43. KORNETSKY, C. 1985. Brain-stimulation reward: A model for the neuronal bases for drug-induced euphoria. NIDA Res. Monogr. 62: 30–50.

44. DI CHIARA, G. & A. IMPERATO. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85: 5274–5278.

45. HARRIS, R. T., W. WATERS & D. McLENDON. 1974. Evaluation of reinforcing capability of Δ⁹-tetrahydrocannabinol in rhesus monkeys. Psychopharmacologia 37: 23–29.

46. LEITE, J. R. & E. A. CARLINI. 1974. Failure to obtain "cannabis-directed behavior" and abstinence syndrome in rats chronically treated with cannabis sativa extracts. Psychopharmacologia 36: 133–145.

47. STARK, P. & P. B. DEWS. 1980. Cannabinoids. I. Behavioral Effects. J. Pharmacol. Exp. Ther. 214: 124–130.

48. GARDNER, E. L., W. PAREDES, D. SMITH, A. DONNER, C. MILLING, D. COHEN & D. MORRISON. 1988. Facilitation of brain stimulation reward by Δ⁹-tetrahydrocannabinol. Psychopharmacology 96: 142–144.

49. GARDNER, E. L., J. CHEN, W. PAREDES, J. LI & D. SMITH. 1989. Strain-specific facilitation of brain stimulation reward by Δ⁹-tetrahydrocannabinol in laboratory rats is mirrored by strain-specific facilitation of presynaptic dopamine efflux in nucleus accumbens. Soc. Neurosci. Abstr. 15: 638.

50. MILLER, L. L. 1984. Marijuana: Acute effects on human memory. In The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. S. Agurell, W. L. Dewey & R. E. Willette, Eds.: 21–46. Academic Press. New York.

51. DOUGLAS, R. J. 1967. The hippocampus and behavior. Psychol. Bull. 67: 416–442.

52. EICHENBAUM, H. & N. J. COHEN. 1988. Representation in the hippocampus: What do hippocampal neurons encode? Trends Neurosci. 11: 244–248.

53. HOOKER, W. D. & R. T. JONES. 1987. Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. Psychopharmacology 91: 20–24.

54. SCHULZE, G. E., D. E. McMILLAN, J. R. BAILEY, A. SCALLET, S. F. ALI, W. J. SLIKKER & M. G. PAULE. 1988. Acute effects of Δ-9-tetrahydrocannabinol in rhesus monkeys as measured by performance in a battery of complex operant tests. J. Pharmacol. Exp. Ther. 245: 178–186.

55. CAMPBELL, K. A., T. C. FOSTER, R. E. HAMPSON & S. A. DEADWYLER. 1986. Effects of Δ⁹-tetrahydrocannabinol on sensory-evoked discharges of granule cells in the dentate gyrus of behaving rats. J. Pharmacol. Exp. Ther. 239: 941–945.

56. MARSDEN, C. D. 1981. Treatment of torsion dystonia. In Disorders of Movement, Current Status of Modern Therapy, Vol. 8. A. Barbeau, Ed.: 81–104. Lippincott. Philadelphia.

57. PETRO, D. J. & C. E. ELLENBERGER. 1981. Treatment of human spasticity with delta-9-tetrahydrocannabinol. J. Clin. Pharmacol. 21: 413s–416s.

58. CLIFFORD, D. B. 1983. Tetrahydrocannabinol for tremor in multiple sclerosis. Ann. Neurol. 13: 669–671.

59. MEINCK, H.-M., P. W. SCHONLE & B. CONRAD. 1989. Effect of cannabinoids on spasticity and ataxia in multiple sclerosis. J. Neurol. 236: 120–122.