Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1932–1936, March 1990
Neurobiology

Cannabinoid receptor localization in brain

(tetrahydrocannabinol/autoradiography/basal ganglia/hippocampus/cerebellum)

MILES HERKENHAM*†, ALLISON B. LYNN*, MARK D. LITTLE*, M. ROSS JOHNSON‡, LAWRENCE S. MELVIN§, BRIAN R. DE COSTA¶, AND KENNER C. RICE¶

*Unit on Functional Neuroanatomy, Building 36, Room 2D-15, National Institute of Mental Health, Bethesda, MD 20892; ‡Glaxo Inc., Research Triangle Park, NC 27709; §Central Research, Pfizer Inc., Groton, CT 06340; and ¶Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892

Communicated by Walle J. H. Nauta, December 7, 1989

ABSTRACT **[^{3}H]CP 55,940, a radiolabeled synthetic cannabinoid, which is 10–100 times more potent *in vivo* than Δ^9-tetrahydrocannabinol, was used to characterize and localize a specific cannabinoid receptor in brain sections. The potencies of a series of natural and synthetic cannabinoids as competitors of [^{3}H]CP 55,940 binding correlated closely with their relative potencies in several biological assays, suggesting that the receptor characterized in our *in vitro* assay is the same receptor that mediates behavioral and pharmacological effects of cannabinoids, including human subjective experience. Autoradiography of cannabinoid receptors in brain sections from several mammalian species, including human, reveals a unique and conserved distribution; 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. Generally high densities in forebrain and cerebellum implicate roles for cannabinoids in cognition and movement. Sparse densities in lower brainstem areas controlling cardiovascular and respiratory functions may explain why high doses of Δ^9-tetrahydrocannabinol are not lethal.**

Marihuana (*Cannabis sativa*) is one of the oldest and most widely used drugs in the world (1, 2). The major psychoactive ingredient of the marihuana plant is Δ^9-tetrahydrocannabinol (Δ^9-THC) (3). Δ^9-THC and other natural and synthetic cannabinoids produce characteristic motor, cognitive, and analgesic effects (4, 5). Early reports showing cannabinoid-like activity of 9β-hydroxyhexahydrocannabinol (β-HHC) (6–8) inspired the synthesis of several distinct cannabinoids for studies of their potential use as analgesics (9). 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. One of these, CP 55,940, was tritiated and used to identify and fully characterize a unique cannabinoid receptor in membranes from rat brain (10). In this study we characterize and validate the binding of [^{3}H]CP 55,940 in slide-mounted brain sections and use the same assay conditions to autoradiographically visualize the distribution of cannabinoid receptors.

METHODS

[^{3}H]CP 55,940 is a bicyclic molecule that is one of a series of synthetic cannabinoids whose structure and biological activity have been documented (9–12) (Fig. 1). It was custom radiolabeled at DuPont/NEN by tritium reduction of CP 60,106 (10). The product was purified by thin layer chromatography on silica gel, eluting with ethylacetate/hexane [1:9 (vol/vol)], and the band comigrating with unlabeled CP 55,940 was extracted, giving a radiochemical yield of 15% and a specific activity of 79 Ci/mmol (1 Ci = 37 GBq). Optimization and competition studies were carried out with slide-mounted sections cut from unfixed frozen rat brains. Incubations were in plastic cytomailers (CMS), each containing eight 30-μm-thick "sausage" sections on four gelatin-coated slides in 5 ml of solution (13). The sausage sections were prepared by combining and mincing three whole rat brains to achieve relative homogeneity of receptor and protein con-

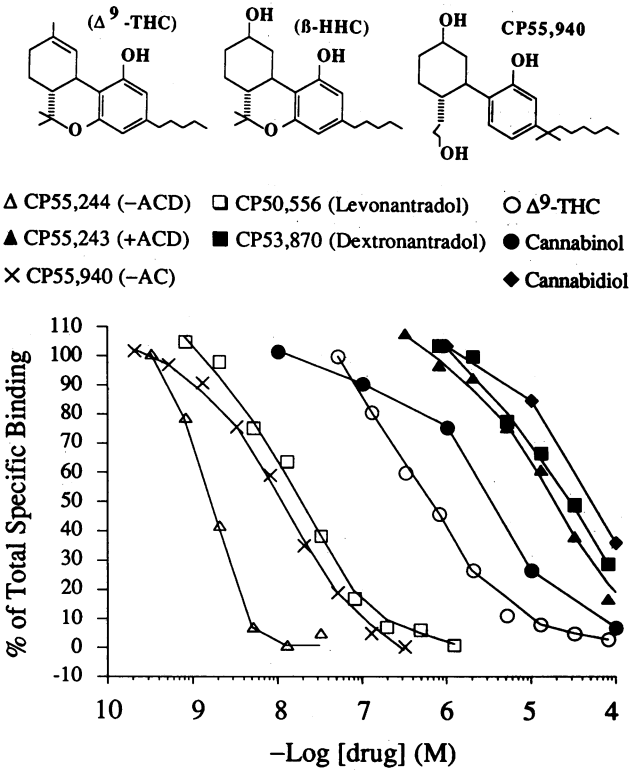

FIG. 1. (*Upper*) Structures of Δ^9-THC (the active ingredient of marihuana), β-HHC (the original synthetic cannabinoid from which the CP compounds were derived), and CP 55,940. (*Lower*) Competitive inhibition of 1 nM [^{3}H]CP 55,940 binding in whole rat brain sausage sections by various synthetic and natural cannabinoids at the concentrations indicated. The data are normalized to specific binding (total minus nonspecific binding) in the absence of competitors. Nonspecific binding was determined by addition of 10 μM CP 55,244 [the most potent cannabinoid in the CP series (9)] and typically represented 10–20% of total binding at both 1 and 10 nM [^{3}H]CP 55,940. Data points represent means of eight determinations. ACD, tricyclic; AC, bicyclic ring nomenclature of Johnson and Melvin (9).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Δ^8- and Δ^9-THC, Δ^8- and Δ^9-tetrahydrocannabinol, respectively; BSA, bovine serum albumin; HHC, hydroxyhexahydrocannabinol; SNr, substantia nigra pars reticulata.

†To whom reprint requests should be addressed.

tent, then placing the paste into a tube, and freezing it to produce a cylindrical sausage that can be cryostat-cut to make sections of uniform composition and size (14). Accuracy of dilutions was checked in mock-incubations in which either [^{3}H]CP 55,940 or [^{3}H]Δ^9-THC (provided by the National Institute on Drug Abuse) were substituted for unlabeled drug, and the solutions were assayed for radioactivity.

To determine binding kinetics, two concentrations of [^{3}H]CP 55,940 (1 and 10 nM) were each competitively inhibited by 6–12 concentrations of unlabeled drug. Competitive inhibition curves were subjected to binding surface analysis, which is a computerized iterative curve-fitting program for determining best-fit parameter estimates (K_d, K_i, and B_{max}) according to 1- or 2-site competitive binding models (15–17). Determination of fmol bound per section was by liquid scintillation counting of the section-laden slide fragments placed overnight in detergent fluor. Some sausage sections were analyzed for protein content by the method of Lowry *et al.* (18) and found to have 456 ± 26 μg of protein per section.

Autoradiography was performed on 25-μm-thick brain sections of rat (male Sprague–Dawley, n = 12), guinea pig (male Hartley, n = 4), dog (beagle, n = 2), rhesus monkey (n = 1), and human (dying of nonneurological disorders, n = 3). Sections were incubated in 10 nM [^{3}H]CP 55,940 by using optimized conditions, washed, dried, and exposed to tritium-sensitive film (LKB or Amersham) for 3–4 weeks before developing.

RESULTS

Assay conditions yielding 80–90% specific binding were as follows: incubation at 37°C for 2 hr in 50 mM Tris·HCl (pH 7.4) containing 5% (wt/vol) bovine serum albumin (BSA) and 1–10 nM [^{3}H]CP 55,940 and washing at 0°C for 4 hr in the same buffer with 1% BSA. By using these optimized conditions, the sausage studies showed that binding was saturable and that competitive inhibition curves were best-fit by a single-site kinetic model: the affinity (K_d) of [^{3}H]CP 55,940 was 15 ± 3 nM and the capacity (B_{max}) in whole brain was 0.9 pmol/mg of protein. Similar parameters were obtained if 1% BSA was used in the incubation, but variability was greater. Binding of 1 nM [^{3}H]CP 55,940 was completely blocked by 10 μM Δ^9-THC, which showed inhibition in a dose-dependent fashion (Fig. 1).

Table 1. Relative potencies of cannabinoid analogs in *in vitro* and *in vivo* animal and human experiments

Drug	K_i, nM	Dog ataxia, mg/kg	Mouse catalepsy, mg/kg	Mouse spontaneous activity, mg/kg	Mouse analgesia, mg/kg: Tail flick	Hot plate	Writhing	Cyclase inhibition, nM	Inhibition of ileal contractions, nM	Human high: % Δ^9-THC	Human high: mg
CP 55,940 (−AC)	15 ± 3 (K_d)		0.35	0.04	0.09	0.7	0.06	25			
CP 56,667 (+AC)	470 ± 57		>10	3	6		15	>5,000			
CP 55,244 (−ACD)	1.4 ± 0.3		0.085	0.004	0.01	0.09	0.02	5			
CP 55.243 (+ACD)	18,000 ± 1100		>10	8	>10		>100	>10,000			
CP 50,556	14 ± 2		1.5	0.1	0.3	0.4	0.07	100	10	400	0.5
CP 53,870	26,000 ± 3500		>10	>10	>10		6.5	>5,000			
CP 54,939	14 ± 2	0.05			0.7		0.06	7			
Nabilone	120 ± 13	0.03		2.5					100	500	1
β-HHC	124 ± 17	0.1		2.5		1.6					
α-HHC	2,590 ± 360	0.5		5.0		>50					
(−)-Δ^9-THC	420 ± 51	0.5	1.6	3.1	1.3	10	5.9	430	100	100	1
(+)-Δ^9-THC	7,700 ± 2100	>2.0	>75	14.6		>100			>2000		
Δ^8-THC	498 ± 52	0.5	0.5	10		8.8			100	75	2
11-OH-Δ^9-THC	210 ± 56	0.05		1.2		1.9			15	120	1
TMA-Δ^8-THC	2,300 ± 1000								1000		
8β-OH-Δ^9-THC	4,200 ± 700								1000	20	10
8α-OH-Δ^9-THC	8,700 ± 1800								3500	25	10
11-OH-Cannabinol	800 ± 150								<1000		
Cannabinol	3,200 ± 450			83					>2000	0	>15
Cannabidiol	53,000 ± 6700	Inactive	25	83		>100			>2000	0	>30
Cannabigerol	275,000	>7.0							>2000	0	
9-COOH-11-nor-Δ^9-THC	75,000		>40			10					
9-COOH-11-nor-Δ^8-zTHC	Inactive			>40			20				
Regression on K_i:R^2		0.96	0.40	0.34	0.78	0.69	0.26	0.54	0.44	0.90	
Significance (2-tailed)		$P < 0.0001$	$P < 0.03$	$P < 0.03$	$P < 0.005$	$P < 0.001$	$P > 0.05$	$P < 0.05$	$P < 0.03$	$P < 0.0001$	

CP analogs were synthesized at Pfizer Central Research; their structures are given in Johnson and Melvin (9). The first six analogs are enantiomeric pairs. Nabilone was a gift of Lilly Research Laboratories. β-HHC was provided by May's group (6–8). The remaining analogs were provided by the National Institute on Drug Abuse. The last two drugs are Δ^9-THC metabolites. The K_i values (mean ± SD) were derived from binding surface analysis of data from the sausage-section binding assay (13, 17). The potencies in other tests, given as 50% of the effective dose or maximum possible effect, are from the literature as follows: dog ataxia, mouse catalepsy, spontaneous activity, and analgesia (6–9, 12, 19); cyclase inhibition (11); inhibition of guinea pig ileal contractions (20); human high (subjective rating, not connoting either pleasantness or unpleasantness; values are: potencies relative to Δ^9-THC, and mg p.o. or i.v. per dose per subject) (3). R^2 values were determined by least squares linear regression analysis. Drugs that show no inhibition of [^{3}H]CP 55,940 binding at 10 μM concentration are as follows: amphetamine, β-estradiol, *cis*-flupenthixol, cocaine, corticosterone, cyclohexyl-adenosine, dexamethasone, etorphine, γ-aminobutyric acid, glutamate, leukotriene B_4 and D_4 (both at 1 μM), lysergic acid diethylamide (LSD), phencyclidine (PCP), prostaglandin E_2, and Ro 15-1788. The significance was determined relative to K_i values. ACD and AC, ring nomenclature (9). TMA, trimethylammonium. CP 50,556 is levonantradol; CP 53,870 is dextronantradol; CP 54,939 is desacetyl levonantradol.

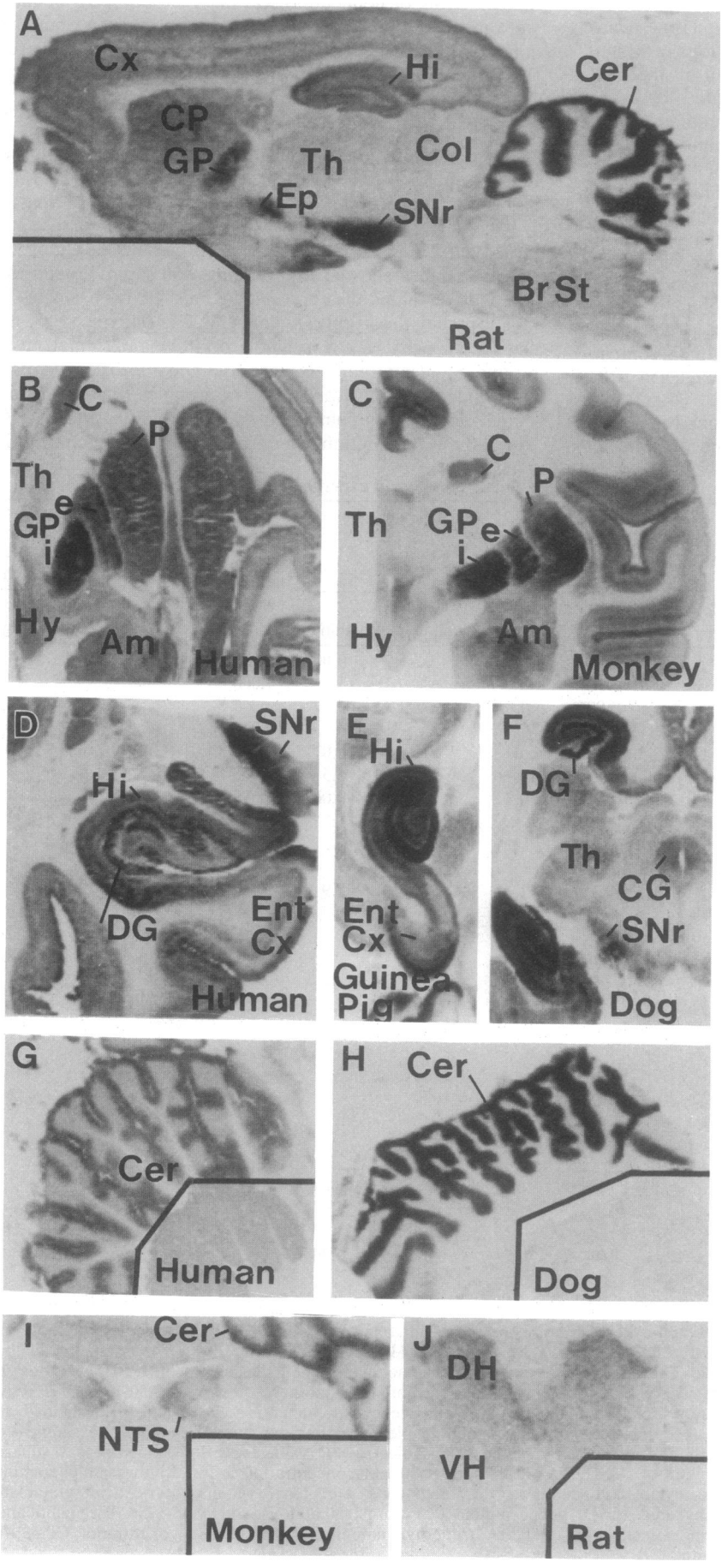

FIG. 2. Autoradiography of 10 nM [^{3}H]CP 55,940 binding in brain. Tritium-sensitive film was exposed for 4 weeks, developed, and computer digitized. Images were photographed directly from the computer monitor. Gray levels represent relative levels of receptor densities. Sagittal section of rat brain is in *A* (×4.7). Coronal brain sections of human are in *B* (×1.3), *D* (×1.7), and *G* (×2.6); rhesus monkey is in *C* (×1.8) and *I* (×4.8); dog is in *F* (×2.1) and *H* (×2.6); and rat is in *J* (×13). Horizontal section of guinea pig brain is in *E* (×4.1). *Insets* in *A* and *G–J* show nonspecific binding in adjacent sections. Miniaturized images are shown. Nonspecific binding accounted for 5% of the total binding in densely labeled structures and all of the binding in the most sparsely labeled structures. Am, amygdala; Br St, brainstem; 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; Th, thalamus; VH, ventral horn of spinal cord.

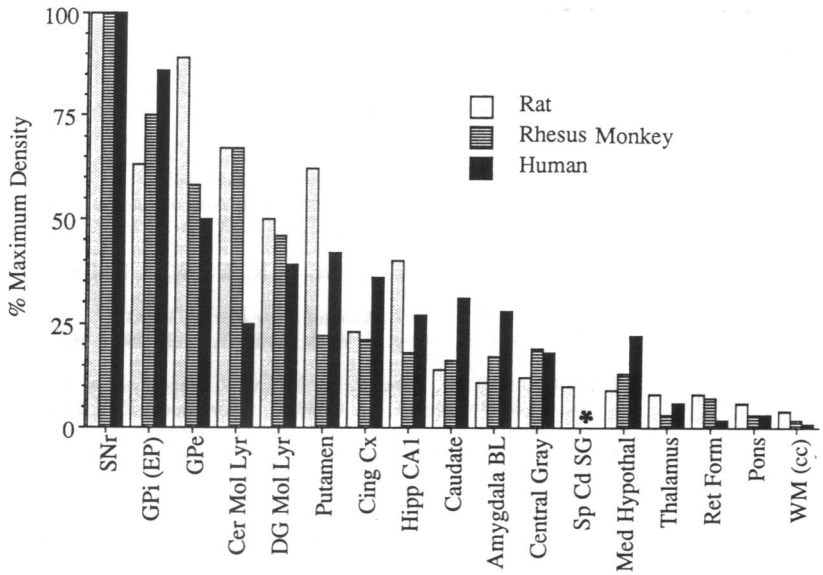

FIG. 3. Relative densities of cannabinoid receptors across brain structures in rat, rhesus monkey, and human. Autoradiographic images were digitized by a solid state video camera and Macintosh II computer-based system for quantitative densitometry using IMAGE software (Wayne Rasband, Research Services Branch, National Institute of Mental Health). Transmittance levels were converted to fmol/mg of tissue by using tritium standards (Amersham high-density microscales) and then normalized to the most dense 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 Fig. 2 legend are as follows: 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.

Inhibition by other natural and synthetic cannabinoids was also shown (Fig. 1; for K_i values, see Table 1).

The data from the section-binding assay were in close agreement with data from a centrifugation assay using membranes from rat cortex (10). The B_{max} was similar in the two studies (though ours was derived from whole brain), but the K_d in our assay was about 100-fold higher. The low affinity in sections relative to that in membranes appears to reflect differences in the nature of the assays. In both assays the addition of guanine nucleotides converted the receptor to a low-affinity state. In sections the nonhydrolyzable GTP analog, guanosine 5′-[β,γ-imido]triphosphate, at 10 μM inhibited binding of 10 nM [^{3}H]CP 55,940 by 94%, and the GDP analog, guanosine 5′-[β-thio]diphosphate, at 10 μM inhibited binding by 79%. Finally, in both assays there was a similar rank order of drug potencies.

For several cannabinoids, inhibition constants (K_i values) and relative biological potencies are given in Table 1. Highly significant correlations exist between the K_i values and potencies of the drugs in tests of dog ataxia and human subjective experience, the two most reliable markers of cannabinoid activity (4, 5). Correlations with potencies in the other tests suggest that the measured effects were similarly receptor-mediated. Enantioselectivity was striking; the (−) and (+) forms of CP 55,244 differed by more than 10,000-fold *in vitro*, a separation predicted by the rigid structure of the molecule (9) and by potencies *in vivo*. Natural cannabinoids lacking psychoactive properties, such as cannabidiol, showed extremely low potency at the receptor, and all tested noncannabinoid drugs had no potency (Table 1).

Autoradiography showed that in all species very dense binding was 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 was also found in the cerebral cortex, other parts of the hippocampal formation, and striatum. In rat, rhesus monkey, and human, the SNr contained the highest level of binding (Fig. 3). In dog, the cerebellar molecular layer was most dense (Fig. 2*H*). In guinea pig and dog, the hippocampal formation had selectively dense binding (Fig. 2 *E* and *F*). Neocortex in all species had moderate binding across fields, with peaks in superficial and deep layers. Very low and homogeneous binding characterized 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—showed slightly higher but still sparse binding (Figs. 2 and 3).

Quantitative autoradiography confirmed the very high numbers of receptors, exceeding 1 pmol/mg of protein in densely labeled areas (data not shown). Cannabinoid receptor density was far in excess of densities of neuropeptide receptors and was similar to levels of cortical benzodiazepine (21), striatal dopamine (22, 23), and whole-brain glutamate receptors (24).

DISCUSSION

Previous attempts to characterize the cannabinoid receptor were unsuccessful for several reasons (for discussion, see ref. 10). Cannabinoids are extremely hydrophobic and adhere to filters (see ref. 10) and other surfaces (25). The section assay circumvents some of these problems; in addition, 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. Other obstacles were the use of Δ^8-[^{3}H]THC (26) or Δ^9-[^{3}H]THC (27), which bind with low affinity and have low specific activities, or the use of 5′-[^{3}H]trimethylammonium-Δ^8-THC (20), which does not act like a cannabinoid in most animal tests and which has low affinity for the presently described receptor (Table 1). In contrast, [^{3}H]CP 55,940 has high specific activity, high affinity, and biological activity similar to that of Δ^9-THC.

The structure–activity profile suggests that the receptor defined by the binding of [^{3}H]CP 55,940 is the same receptor that mediates all of the behavioral and pharmacological effects of cannabinoids listed in 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 (Table 1). There was no compelling evidence for receptor subtypes from the present analysis.

The overall central nervous system distribution, although not similar to any known drug or neurotransmitter receptor pattern, resembles autoradiographic distributions of second messengers (28, 29). These mapping similarities, the very high abundance of the cannabinoid receptor, and the profound inhibition of binding by guanine nucleotides suggest that the cannabinoid receptor is closely associated with second messenger systems. Total inhibition of binding by the GTP analog indicates that the receptor is functionally and

strongly coupled to a guanine nucleotide-binding regulatory (G) protein in our assay. It also indicates that the ligand is an agonist and that there are multiple affinity states of the receptor, as found with the other major receptor classes coupled to adenylate cyclase by G proteins (30).

Dense binding in the basal ganglia and cerebellum suggests cannabinoid involvement in movement control. Cannabinoids depress motor functions with a characteristic stimulatory component (4, 5). Dog shows a static ataxia (Table 1) and has high receptor levels in cerebellum and relatively low levels in SNr (Fig. 2 *F* and *H*). Human shows much less motor depression (3–5) and lower relative densities in cerebellum (Fig. 3), suggesting cerebellar mediation of the motor impairments in animals.

Accounts of cannabis use in humans stress the loosening of associations, fragmentation of thought, and confusion on attempting to remember recent occurrences (5, 31). The most consistent effect of Δ^9-THC on performance is disruption of selective aspects of short-term memory tasks, similar to that found in monkeys and patients with damage to limbic cortical areas (31–33). These cognitive effects may be mediated by receptors in the cerebral cortex. The hippocampal cortex "gates" information during memory consolidation and codes spatial and temporal relations among stimuli and responses (34, 35). Δ^9-THC causes memory "intrusions" (36), impairs temporal aspects of performance (37), and suppresses hippocampal electrical activity (38).

The presence of cannabinoid receptors in the ventromedial striatum suggests an association with dopamine circuits thought to mediate reward (39–41). However, reinforcing properties of cannabinoids have been difficult to demonstrate in animals (42, 43). Moreover, cannabinoid receptors in the basal ganglia are not localized on dopamine neurons (44).

There are virtually no reports of fatal cannabis overdose in humans (1, 4, 5). The safety reflects the paucity of receptors in medullary nuclei that mediate respiratory and cardiovascular functions.

Anticonvulsant and antiemetic effects of cannabinoids have therapeutic value (4, 5). The localization of cannabinoid receptors in motor areas suggests additional therapeutic applications. Cannabinoids exacerbate hypokinesia in Parkinson disease but are beneficial for some forms of dystonia, tremor, and spasticity (4, 5, 45–47). The development of an antagonist could provide additional therapeutic uses of value. The receptor binding assay will be helpful in this regard, and it can be used also to screen drugs that have greater potency or bind irreversibly to aid in the identification of the receptor gene and the putative endogenous ligand.

We thank Drs. J. Atack, J. Hill, and J. Johannessen for providing human and dog tissues, Dr. R. Rothman for pharmacokinetics advice, and Drs. A. Howlett and W. Devane for sharing unpublished data.

1. Harris, L. S., Dewey, W. L. & Razdan, R. K. (1977) in *Handbook of Experimental Pharmacology*, ed. Martin, W. R. (Springer, New York), pp. 371–429.
2. Mechoulam, R. (1986) in *Cannabinoids as Therapeutic Agents*, ed. Mechoulam, R. (CRC, Boca Raton, FL), pp. 1–19.
3. Razdan, R. K. (1986) *Pharmacol. Rev.* **38**, 75–149.
4. Dewey, W. L. (1986) *Pharmacol. Rev.* **38**, 151–178.
5. Hollister, L. E. (1986) *Pharmacol. Rev.* **38**, 1–20.
6. Wilson, R. S. & May, E. L. (1975) *J. Med. Chem.* **18**, 700–703.
7. Wilson, R. S., May, E. L., Martin, B. R. & Dewey, W. L. (1976) *J. Med. Chem.* **19**, 1165–1167.
8. Wilson, R. S., May, E. L. & Dewey, W. L. (1979) *J. Med. Chem.* **22**, 886–888.
9. Johnson, M. R. & Melvin, L. S. (1986) in *Cannabinoids as Therapeutic Agents*, ed. Mechoulam, R. (CRC, Boca Raton, FL), pp. 121–145.
10. Devane, W. A., Dysarz, F. A. I., Johnson, M. R., Melvin, L. S. & Howlett, A. C. (1988) *Mol. Pharmacol.* **34**, 605–613.
11. Howlett, A. C., Johnson, M. R., Melvin, L. S. & Milne, G. M. (1988) *Mol. Pharmacol.* **33**, 297–302.
12. Little, P. J., Compton, D. R., Johnson, M. R. & Martin, B. R. (1988) *J. Pharmacol. Exp. Ther.* **247**, 1046–1051.
13. Herkenham, M. (1988) in *Molecular Neuroanatomy*, eds. van Leeuwen, F., Buijs, R. M., Pool, C. W. & Pach, O. (Elsevier, Amsterdam), pp. 111–120.
14. Rothman, R. B., Schumacher, U. K. & Pert, C. B. (1983) *Neuropeptides* **3**, 493–499.
15. Rothman, R. B. (1986) *Alcohol Drug Res.* **6**, 309–325.
16. McGonigle, P., Neve, K. A. & Molinoff, P. B. (1986) *Mol. Pharmacol.* **30**, 329–337.
17. Rothman, R. B., Long, J. B., Bykov, V., Jacobson, A. E., Rice, K. C. & Holaday, J. W. (1988) *J. Pharmacol. Exp. Ther.* **247**, 405–416.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
19. Burstein, S. H., Hull, K., Hunter, S. A. & Latman, V. (1988) *FASEB J.* **2**, 3022–3026.
20. Nye, J. S., Seltzman, H. H., Pitt, C. G. & Snyder, S. H. (1985) *J. Pharmacol. Exp. Ther.* **234**, 784–791.
21. Zezula, J., Cortés, R., Probst, A. & Palacios, J. M. (1988) *Neuroscience* **25**, 771–795.
22. Boyson, S. J., McGonigle, P. & Molinoff, P. B. (1986) *J. Neurosci.* **6**, 3177–3188.
23. Richfield, E. K., Young, A. B. & Penney, J. B. (1986) *Brain Res.* **383**, 121–128.
24. Greenamyre, J. T., Young, A. B. & Penney, J. B. (1984) *J. Neurosci.* **4**, 2133–2144.
25. Garrett, E. R. & Hunt, C. A. (1974) *J. Pharm. Sci.* **63**, 1056–1064.
26. Harris, L. S., Carchman, R. A. & Martin, B. R. (1978) *Life Sci.* **22**, 1131–1138.
27. Roth, S. H. & Williams, P. J. (1979) *J. Pharm. Pharmacol.* **31**, 224–230.
28. Worley, P. F., Baraban, J. M., De Souza, E. B. & Snyder, S. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4053–4057.
29. Worley, P. F., Baraban, J. M. & Snyder, S. H. (1986) *J. Neurosci.* **6**, 199–207.
30. Dohlman, H. G., Caron, M. G. & Lefkowitz, R. J. (1987) *Biochemistry* **26**, 2257–2264.
31. Miller, L. L. (1984) in *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects*, eds. Agurell, S., Dewey, W. L. & Willette, R. E. (Academic, New York), pp. 21–46.
32. Miller, L. L. & Braconnier, R. J. (1983) *Psychol. Bull.* **93**, 441–456.
33. Aigner, T. G. (1988) *Psychopharmacology* **95**, 507–511.
34. Douglas, R. J. (1967) *Psychol. Bull.* **67**, 416–442.
35. Eichenbaum, H. & Cohen, N. J. (1988) *Trends Neurosci.* **11**, 244–248.
36. Hooker, W. D. & Jones, R. T. (1987) *Psychopharmacology* **91**, 20–24.
37. Schulze, G. E., McMillan, D. E., Bailey, J. R., Scallet, A., Ali, S. F., Slikker, W. J. & Paule, M. G. (1988) *J. Pharmacol. Exp. Ther.* **245**, 178–186.
38. Campbell, K. A., Foster, T. C., Hampson, R. E. & Deadwyler, S. A. (1986) *J. Pharmacol. Exp. Ther.* **239**, 941–945.
39. Kornetsky, C. (1985) *NIDA Res. Monogr.* **62**, 30–50.
40. Di Chiara, G. & Imperato, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5274–5278.
41. Ng Cheong Ton, J. M., Gerhardt, G. A., Friedemann, M., Etgen, A. M., Rose, G. M., Sharpless, N. S. & Gardner, E. L. (1988) *Brain Res.* **451**, 59–68.
42. Stark, P. & Dews, P. B. (1980) *J. Pharmacol. Exp. Ther.* **214**, 124–130.
43. Gardner, E. L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D. & Morrison, D. (1988) *Psychopharmacology* **96**, 142–144.
44. Herkenham, M., Lynn, A. B., de Costa, B. & Richfield, E. K. (1989) *Soc. Neurosci. Abstr.* **15**, 905.
45. Marsden, C. D. (1981) in *Disorders of Movement, Current Status of Modern Therapy*, ed. Barbeau, A. (Lippincott, Philadelphia), Vol. 8, pp. 81–104.
46. Petro, D. J. & Ellenberger, C. E. (1981) *J. Clin. Pharmacol.* **21**, 413s–416s.
47. Clifford, D. B. (1983) *Ann. Neurol.* **13**, 669–671.