# The cannabinoid receptor: biochemical, anatomical and behavioral characterization

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*The actions of the active principle of marihuana, $\Delta^9$-tetrahydrocannabinol, are mimicked by synthetic cannabinoid agonists showing high potency and enantioselectivity in behavioral assays. These drugs have been used to characterize cannabinoid receptor binding, biochemistry and pharmacology, leading to a better understanding of the effects of cannabinoids in the CNS of humans and experimental animals.*

$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC) has been recognized as the major psychoactive component of marihuana for over 25 years[1]. However, until recently a mechanism by which $\Delta^9$-THC and other natural and synthetic psychoactive cannabinoids produce their effects *in vivo* was not readily apparent. Because the cellular and biochemical mechanisms of action of psychoactive cannabinoids were not understood, neuroscientists were allowed great breadth to speculate upon the influence that these compounds might have on neurons in the brain. Over the past two decades, a substantial multidisciplinary research effort has focused on examining the effects of cannabinoid compounds on biological and synthetic membranes, ATPase and monoamine oxidase activities, eicosanoid metabolism, hormone and neurotransmitter receptor binding, and synaptosomal uptake of neurotransmitters[2,3]. Most of the *in vitro* cannabinoid actions required concentrations of $\Delta^9$-THC that were greater than estimated brain concentrations coincident with biological responses following *in vivo* administration[4,5]. Furthermore, many *in vitro* effects of cannabinoids failed to show a structure–activity relationship consistent with the *in vivo* behavioral pharmacology[2,3]. Thus, it was difficult to attribute the behavioral effects of $\Delta^9$-THC to any single *in vitro* effect.

Significant progress has been made in the cannabinoid field in recent years by using a class of high-potency synthetic compounds originally developed for their analgetic properties[6]. These compounds are typified by levonantradol, its active metabolite desacetyllevonantradol, and CP-55 940 (Fig. 1). This review describes the recent efforts to elucidate the neurochemical properties and neuroanatomical localization of a unique receptor for these cannabinoid compounds and speculates on the role that this receptor plays in the effects of cannabinoid compounds on behavior.

## Cannabinoid pharmacology and the cAMP second messenger system

Several animal models are currently used to study cannabinoid drug effects *in vivo*. Assessments of cannabinoid activity and structure–activity relationships for over 100 classical cannabinoids have been compiled by Razdan[7]. Evidence suggests that a number of the characteristic behavioral effects of cannabinoids may result from their interaction with a stereoselective receptor site in the brain. Enantiomers of $\Delta^9$-THC have been shown to differ in potency by ten-to 100-fold in depressing schedule-controlled responding in monkeys or in producing static ataxia in dogs[7,8]. $\Delta^9$-THC and cannabinoid analogs are also active in lowering body temperature and altering spontaneous activity in rodents[8,9]. Levonantradol was up to 30 times, and (–)CP-55 940 was more than 50 times more potent than $\Delta^9$-THC in these studies, whereas the dextro-isomers were poorly active or inactive at the highest concentrations tested[9] (Table I). Rodents also exhibit a typical immobility (or catalepsy) in response to $\Delta^9$-THC and synthetic analogs (Table I)[9].

*In vitro* studies using cultured neuroblastoma cells, neuroblastoma × glioma hybrid cells and brain slice preparations demonstrated that one cellular action of cannabinoid drugs is the reversible inhibition of cAMP production[10–12]. Further studies demonstrated that psychoactive cannabinoid compounds inhibit adenylate cyclase activity via the G protein, $G_i$, suggesting a receptor-coupled mechanism[10,12,13]. The inhibition of adenylate cyclase by natural and synthetic cannabinoids is enantioselective, and the pharmacological profile for regulation of adenylate cyclase correlates well with that observed for several animal models of cannabinoid activity[14,15]. These findings suggest that the receptor characterized in cell lines is the same as the receptor responsible for certain cannabinoid actions in the CNS[9,14,15] (Table I).

It is certainly possible that multiple signal transduction mechanisms may be found to be linked to cannabinoid actions in the CNS. Arachidonic acid accumulation in response to cannabinoid compounds has been demonstrated in brain slice preparations[16]. However, studies suggest that this is not due to a receptor-regulated phospholipase C or phospholipase A2, but rather to the inhibition of arachidonic acid acylation on membrane lipids[17].

## Localization and functional significance of cannabinoid receptors in the brain

Synthesis of a potent radiolabeled ligand, [$^3$H]CP-55 940, led to the development of membrane homogenate and tissue section binding assays for the characterization and localization of the cannabinoid receptor in brain[18,19] (Devane, W. A., PhD Thesis, St Louis University, 1989). The [$^3$H]CP-55 940 binding site is saturable, has high affinity and enantioselectivity for agonist ligands, and exhibits characteristics expected for a neuromodulator receptor associated with a G protein[18,19]. As shown in Table I, the relative potencies with which cannabinoid compounds inhibit

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[$^3$H]CP-55 940 binding parallels the abilities of these compounds to produce behavioral effects in animals[18,19]. A similar structure–activity profile exists for receptor binding and the regulation of adenylate cyclase *in vitro* (Table I)[18,19]. High-affinity ligands for a variety of neurotransmitter, neuromodulator and hormonal classes, including adrenergic, cholinergic, dopaminergic, serotonergic, opioid, GABAergic, glutamatergic, steroid, and prostanoid agonists and antagonists, fail to displace [$^3$H]CP-55 940 binding at this receptor[12,18]. [$^3$H]CP-55 940 binding is also found in the nervous systems of lower vertebrate species (Table II; Devane, W. A., PhD Thesis, St Louis University, 1989). The cannabinoid receptor appears to be conserved across species in that the $K_d$ values are similar, and the non-hydrolysable GTP analog guanylyl-β-γ-imidodiphosphate reduces the binding (Devane, W. A., PhD Thesis, St Louis University, 1989).

It should be noted that a cannabinoid binding site in rat brain homogenates has been described using the ligand [$^3$H]-5′-trimethylammonium $\Delta^8$-THC[20]. It is clear that this binding site is not the same as that for [$^3$H]CP-55 940, because the density of these two sites in various brain regions is not similar, and the structure–activity profile for displacement in equilibrium studies differs. The relative affinities of cannabinoid compounds for the [$^3$H]-5′-trimethylammonium $\Delta^8$-THC binding site does not correlate well with threshold for behavioral responses in the Rhesus monkey[20]. 5′-Trimethylammonium $\Delta^8$-THC does not exhibit biological activity in typical animal behavioral models of cannabinoid action[21], and thus, the pharmacological relevance of this binding site remains in question.

Autoradiography using [$^3$H]CP-55 940 reveals a heterogeneous distribution of cannabinoid receptors throughout the brain (Fig. 2)[18]. A unique pattern of binding is conserved across several mammalian species, including humans, with the greatest abundance of [$^3$H]CP-55 940 binding sites in the basal ganglia, hippocampus and cerebellum[12,18]. In rats, monkeys and humans, the greatest density of cannabinoid receptors is observed in the globus pallidus, the substantia nigra pars reticulata, the molecular layer of the dentate gyrus of the hippocampus, and the cerebellar molecular layer[18]. Receptors are also dense in the cerebral cortex, the neostriatum and in the remainder of the hippocampal formation. Comparatively little binding is observed in the brain stem and spinal cord[12,18].

Current work is addressing the roles of cannabinoid receptors in receptor-rich regions such as the basal ganglia. There is evidence implicating the basal ganglia in the cataleptic response to cannabinoids seen in rodents[22] and in the potentiation by $\Delta^9$-THC and levonantradol of reserpine-induced hypokinesia in a model of Parkinson's disease in rats and primates[23]. Cannabinoid drugs attenuate the $D_1$-dopaminergic stimulation of cAMP production as do $D_2$-dopaminergic agonists and opioid agonists in striatal slices[24]. This suggests that opioid and cannabinoid receptors may be co-localized on the same population of cells that respond to dopamine. Anatomical evidence for this comes from the observation that cannabinoid, $D_1$- and $D_2$-dopaminergic receptors in the striatum, globus pallidus and substantia nigra pars reticulata are lost following ibotinic acid lesions of the striatum[25].

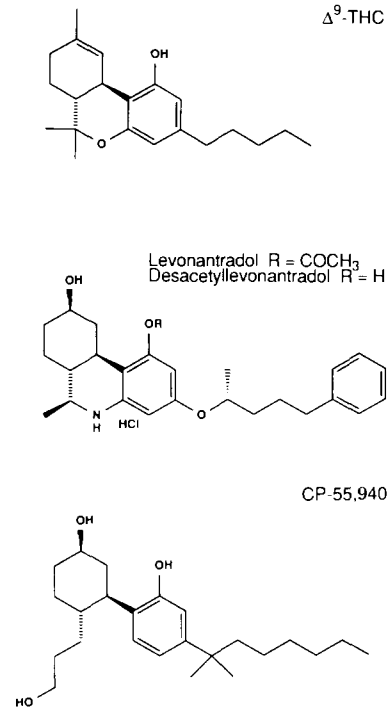


**Fig. 1.** *The chemical structures of $\Delta^9$THC and synthetic cannabinoid compounds.*

Desacetyllevonantradol regulates cAMP production in brain regions exhibiting high receptor binding density. Populations of cells in the cortex, hippocampus and cerebellum, in which the β-adrenergic agonist isoproterenol or vasoactive intestinal peptide stimulate cAMP production, are differentially regulated by cannabinoid drugs[12].

## Actions of cannabinoids in humans

Studies describing the acute and chronic effects of marihuana use in humans have been reviewed recently by Hollister[26]. Much further study is needed to determine the mechanisms and sites of action of the

**TABLE I.** Behavioral and cellular actions of $\Delta^9$-THC and synthetic cannabinoid compounds

| | Antinociception | | Spontaneous activity[b] | Hypothermia[b] | Immobility[b] | Inhibition of adenylate cyclase[c] | Binding to cannabinoid receptor | |
|---|---|---|---|---|---|---|---|---|
| | Phenylbenzyl-quinone writhing[a] | Tail flick[b] | | | | | | |
| | $MPE_{50}$ (mg/kg) | $ED_{50}$ (mg/kg) | $ED_{50}$ (mg/kg) | $ED_{50}$ (mg/kg) | $ED_{50}$ (mg/kg) | $K_{inh}$ (nM) | $K_i$ (nM)[d] | $K_i$ (nM)[e] |
| $\Delta^9$-THC | 5.9 | 1.3 | 3.1 | 24 | 1.6 | 430 | 1600 | 420 |
| Levonantradol | 0.07 | 0.23 | 0.1 | 0.78 | 1.5 | 100 | 1550 | 14 |
| Dextronantradol | 6.5 | >10 | >10 | >10 | >10 | >5000 | >300 000 | 26 000 |
| Desacetyllevonantradol | 0.06 | – | – | – | – | 7 | 123 | 14 |
| (–)CP-55 940 | 0.06 | 0.9 | 0.04 | 0.3 | 0.3 | 25 | 66 | 15 |
| (+)CP-55 940 | 14.6 | 6.0 | 3.0 | >10 | >10 | >5000 | 3400 | 470 |

[a]Ref. 6; drugs administered to mice subcutaneously; $MPE_{50}$ is the dose required to produce 50% of the maximum possible effect.
[b]Ref. 9; drugs administered to mice i.v. in the tail vein.
[c]Refs 13,14; membranes from N18TG2 neuroblastoma cells.
[d]Ref. 16; P2 membranes from rat cortex.
[e]Ref. 15; sections from whole rat brain.

**TABLE II.** Specific binding of [$^3$H]CP-55 940 in CNS homogenates from vertebrate species[a]

| Species | $B_{max}$ (fmol/mg protein) | $K_d$ (pM) |
|---|---|---|
| Chicken *(Gallus gallus)* | 1310 ± 130 | 350 ± 50 |
| Turtle *(C. serpentina)* | 580 ± 170 | 152 ± 19 |
| Frog *(Rana pipiens)* | 1210 ± 450 | 247 ± 77 |
| Trout *(Salmo gairdnerii)*[b] | 650 ± 200 | 190 ± 20 |
| Lamprey *(I. intercostus)*[c] | None detected | |
| *Aplysia (A. californica)*[d] | None detected | |

[a]Values were determined using LIGAND non-linear least squares analysis of homologous displacement equilibrium binding studies, and are the mean ± SEM for three experiments (taken, with permission, from Devane, W. A., PhD Thesis, St Louis University, 1989).
[b]Binding determined at 30°C was poorly detected. The results reported were determined at 15°C.
[c]Binding data from a single experiment performed at 30°C.
[d]Binding was examined at both 30°C and 15°C to homogenates of the buccal and abdominal ganglia.

psychoactive component $\Delta^9$-THC and its analogs in the human brain. One could postulate that the cognitive impairments observed in human users of marihuana, including distractibility, fragmentation of thought and difficulty in solving problems[26,27] may be mediated by cannabinoid receptors in the cortex and hippocampus.

Preparations of *Cannabis sativa* have been used throughout history to relieve pain, and the potential of cannabinoids as analgetics has been discussed in a review by Segal[28]. Levonantradol and its active metabolite desacetyllevonantradol were the first members of a series of synthetic cannabinoid compounds to be extensively studied in rodents and found to be ten to 30 times more potent than morphine across a battery of analgetic tests[6]. The analgetic activity of these compounds has also been demonstrated in humans[6]. The site or sites of the analgetic action in the CNS are currently not established, although concentration of the low levels of receptors in the spinal cord gray matter in the substantia gelatinosa of the dorsal horn suggests an action at the spinal level[18]. Desacetyllevonantradol produces a dose-dependent increase in latencies of behavioral assays of pain, such as hot-plate and tail-flick tests following intrathecal administration in rodents[29]. In addition, cannabinoid drugs inhibit the skin-twitch reflex in spinal dogs[30]. Although these findings suggest a spinal site of action, they do not eliminate the possibility of action at higher levels in the brain. The anti-nociceptive activity of levonantradol and derivatives does not involve opioid receptors, since it is not reversed by high doses of naloxone[31]. Furthermore, these compounds do not bind to μ or δ opioid receptors *in vitro*[11,31]. However, studies using dogs and rodents have shown that $\Delta^9$-THC suppresses abstinence signs following withdrawal of morphine-dependent animals[32,33]. This would suggest that cannabinoid and opioid receptor systems may interact physiologically in the CNS.

Certain neuroendocrine and visceral responses may be influenced by cannabinoids, and this is an area in need of more research. $\Delta^9$-THC is known to decrease serum concentrations of thyrotropin, and to depress gonadotropin and prolactin release in rodents and primates, effects that may result from actions at the hypothalamus or connected structures[34,35]. Evidence indicates that the decrease in body temperature in response to cannabinoid drugs may be mediated in part by depressing thermogenesis at centers in the caudal brain stem as well as by an action at the thermosensory neurons in the anterior hypothalamus and preoptic area[36,37]. Currently, cannabinoid drugs are used therapeutically to ameliorate the nausea and vomiting caused by cancer chemotherapy[38]. This anti-

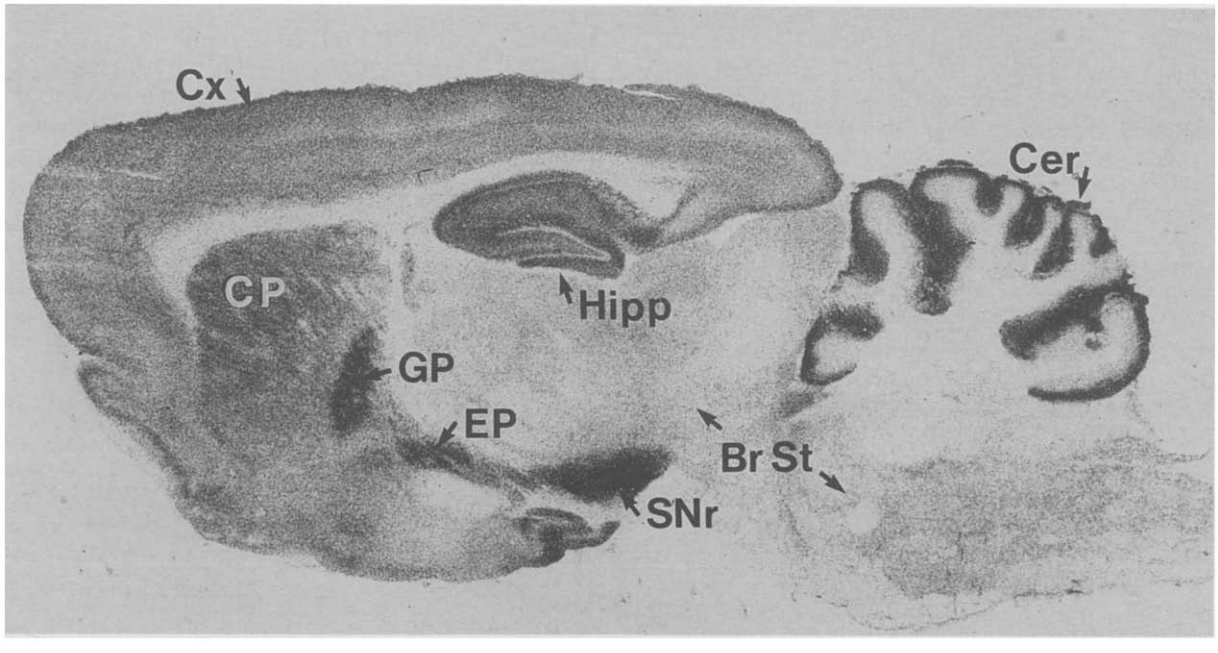


**Fig. 2.** *Autoradiography of cannabinoid receptor distribution in a sagittal section of rat brain. Binding of 10 nM [$^3$H]CP-55 940 is dense in the hippocampus (Hipp), globus pallidus (GP), entopeduncular nucleus (EP), substantia nigra pars reticulata (SNr) and cerebellum (Cer). Binding is moderate in the cerebral cortex (Cx) and caudate-putamen (CP) and sparse in the brain stem (Br St) and spinal cord.*

emetic effect may result from actions affecting the vomiting center in the brain stem or affecting connected structures, such as the amygdala and neocortex, that modulate the activity of the vomiting center[39]. The cardiovascular responses to cannabinoid drugs are complex and vary with species and experimental paradigm[40–42]. In humans, smoking marihuana leads to an increase in heart rate and peripheral blood flow, perhaps due to activation of the sympathetic nervous system or inhibition of parasympathetic influences[40]. In experimental protocols using animals, a slowing of the heart rate is generally observed[41,42].

### A challenge to neuroscientists

Clearly, a plethora of questions about the actions of cannabinoid compounds in the CNS remain. Biochemical, anatomical and neurophysiological studies of the effects of cannabinoid compounds have been limited in the past due to the relatively low potency of $\Delta^9$-THC, its high solubility in membrane lipids, and its tendency to adhere to glass and plastic in *in vitro* experiments (see Refs 2,3). The use of the high-affinity cannabinoid agonists such as desacetyllevonantradol and CP-55 940 in these studies should allow rapid progress in addressing the unique and important actions of cannabinoid compounds in the CNS. Cannabinoid analgetics should be useful in studying novel mechanisms of anti-nociceptive activity at the level of the spinal cord as well as at higher levels in the CNS involved in processing of the response to pain. The anatomical and physiological properties of cannabinoceptive neurons in the hippocampus and cortex must be re-evaluated with respect to the role these neurons may play in cognition and memory. Study of the interactions of cannabinoceptive neurons with afferents to, efferents from, and interneurons within the basal ganglia are clearly warranted. The potential effects of cannabinoid compounds on behaviors and movement disorders associated with the basal ganglia must be re-evaluated. Additionally, the function of cannabinoid receptors in the cerebellum should be addressed. Renewed scientific interest in cannabinoid compounds would stimulate research within a broad range of neuroscience disciplines.

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Note added in proof: In a recent issue of *Nature* (vol. 346, pp. 561–564), L. A. Matsuda *et al.* describe the cloning of a cannabinoid receptor gene from rat cerebral cortex. In the October issue of *Trends in Pharmacological Sciences* Allyn C. Howlett discusses this in a commentary article.