Imaging of the Brain Cannabinoid System

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Abstract This review covers two major strategies for imaging of the brain cannabinoid system: autoradiography and in vivo neuroimaging. Cannabinoid receptors can be imaged directly with autoradiography in brain slices using radiolabeled cannabinoid receptor ligands. In addition, the effects of pharmacologic doses of unlabeled cannabinoid drugs can be autoradiographically imaged using indicators of blood flow or indicators of metabolism such as glucose analogs. Although cannabinoid imaging is a relatively new topic of research compared to imaging of other drugs of abuse, autoradiographic strategies have produced high-quality information about the distribution of brain cannabinoid receptors and the effects

of cannabinoid drugs on brain metabolism. In vivo neuroimaging, in contrast to autoradiography, utilizes noninvasive techniques such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) to image both the binding and the effects of drugs within living brain. These techniques are well developed; however, in vivo imaging of cannabinoid systems is in a very preliminary state. Early results have been promising yet hard to generalize. Definitive answers to some of the most important questions about cannabinoid drugs and their effects await development of suitable in vivo neuroimaging ligands for cannabinoid systems.

Keywords Cannabinoids · Imaging · Metabolism · Blood flow · MRI (magnetic resonance imaging) · PET (positron emission tomography) · Autoradiography

1 Introduction

Tetrahydrocannabinol (Δ^9-THC; the main psychoactive ingredient of cannabis) acts at G protein-coupled receptors (CB_1 receptors) that are abundant in specific brain areas including the cerebellum, hippocampus and outflow nuclei of the basal ganglia. Investigators have imaged these receptors in cryostat sections of brain and also in the living brains of humans and animals. Additionally, the effects of cannabinoid agonists and antagonists on cerebral metabolism and blood flow have been visualized. Two major strategies are reviewed, ex vivo autoradiographical imaging, and in vivo imaging using positron emission tomography (PET), single photon emission computed tomography (SPECT), or magnetic resonance imaging (MRI).

Autoradiography is a relatively old technique, first used in a biological context by Lacassagne in 1924 (Rogers 1973). Radioactivity incorporated into tissues can be used to generate cumulative, spatially accurate representations of the isotope's distribution by placing tissue samples in close proximity to a recording medium, usually, but not always, a photographic emulsion. Due to their invasive nature, autoradiographic strategies necessitate a preclinical or postmortem focus and between-subjects experimental designs.

Over the last quarter century, in vivo imaging modalities have been developed that allow living brains, including the human brain, to be studied in a non-invasive manner. These modalities include PET which utilizes positron emitting radiotracers that are now available for a growing range of neurotransmitter receptors as well as for blood flow and glucose metabolism. PET, as well as the related modality SPECT, have been used to perform the same general kinds of experiments that are possible using ex vivo autoradiography (see Sect. 2). However, in addition, longitudinal and within-subjects experimental designs are possible.

The non-radionuclide modality functional magnetic resonance imaging (fMRI) is also becoming an important tool for human neuropharmacological studies. These include evaluation of acute effects of drugs on control subjects and drug-dependent individuals. Chronic drug use can be evaluated by means of comparing dependent and non-dependent subjects.

Both autoradiographical and in vivo studies of drugs of abuse such as cannabis may help our understanding of mental diseases, and produce useful leads for the development of drug therapies for these illnesses as well as helping us understand mechanisms of addiction. A better understanding of the cannabinoid receptor system might produce more useful therapeutic drugs with less abuse potential.

2 Overview: Five Major Experimental Strategies

Both autoradiography and PET/SPECT neuroimaging are used to visualize the behavior of drug molecules in complex biological systems. Although these techniques are in some ways quite different, they are used to answer the same types of questions by means of the following five types of studies:

1. Biodistribution. Abused substances such as Δ^9-THC can be directly labeled with radioisotopes. This permits quantitative determination and direct measurement of the distribution of drugs throughout the body. This type of information is invaluable when the substrates of drug action are not known. It also has utility when evaluating new synthetic analogs, yielding information about penetration of the blood–brain barrier, and kinetics. Biodistribution studies are discussed in a recent journal special issue (Gatley and Carroll 2003).

2. Receptor mapping. This technique provides highly detailed information about regional drug distribution within a receptor-rich tissue such as the brain.

3. Competition. The ability of an abused drug or therapeutic drug molecule to compete with or to displace a receptor-mapping radioligand for the same binding sites can be measured using imaging techniques. This information allows calculation of the amount of receptor occupancy provided by the given dose of drug. Measurement of the degree of receptor occupancy achieved by a drug potentially allows evaluation of the relationships between receptor occupancy and physiological, behavioral, and subjective effects of the drug.

4. Metabolism and flow. Imaging strategies may be used to measure regional values of cerebral blood flow (rCBF) (Sakurada et al. 1978) and rates of regional cerebral glucose metabolism (rCGM) (Sokoloff et al. 1977). This strategy allows effects of abused drugs on regional and global brain function to be evaluated, since flow and metabolism are correlated with nerve terminal activity.

5. Effects of drugs on diverse neurotransmitter systems. Radioligands, which bind to different sites from the drug of interest, may be used to examine effects of abused drugs on other neurotransmitter systems. For example, the in vivo binding of the dopamine D_2 receptor PET radioligand [^{11}C]raclopride has been shown to be sensitive to alterations in levels of endogenous dopamine (Dewey et al. 1993). Using this technique, the indirect impact of pharmacological doses of a drug of interest, for instance, Δ^9-THC, on other neurotransmitter systems, such

as the dopaminergic system, can be assessed. This strategy has been utilized to date only accidentally in the context of cannabinoid imaging in one human subject discussed in a case report (Voruganti et al. 2001) and is reviewed in Sect. 4.4.

2.1 In Vitro and Ex Vivo Neuroimaging Using Autoradiography

2.1.1 Technique Overview: Autoradiography

In in vitro receptor autoradiography, frozen human or animal brains are processed to form thin (20 μm) sections fixed onto glass slides. The sections are incubated in receptor radioligand solution to allow labeling of receptor-rich areas and washed to remove unbound radioligand. Subsequent exposure of sections using film, imaging plates, or a beta imager yields maps of the receptor distribution. The images produced by autoradiography have spatial resolution of approximately 50 μm, although specialized applications of this technique can yield images with resolution of 0.05 μm. Similar methodology using labeled polynucleotide probes (in situ hybridization) yields maps of gene transcription. In contrast, ex vivo autoradiography involves preparation of postmortem sections after injection of radiotracer into the living animal. Ex vivo autoradiographs have some dependency on physiological factors such as blood flow, as well as on receptor density. Primary foci of research include imaging of labeled cannabinoid receptors directly, and visualization of metabolic effects of cannabinoid drugs via imaging their effects on neuronal metabolism.

2.1.2 Autoradiographic Tracers and Their Substrates

Autoradiographic Mapping of Cannabinoid Receptors Δ^9-THC binds to cannabinoid receptors with only moderate affinity. Synthetic molecules with higher affinities include the non-classical cannabinoid receptor agonist CP 55,940 (Compton et al. 1992b), the aminoalkylindole agonist WIN 55,212-2 (Compton et al. 1992a), and the antagonist SR141716A (Rinaldi-Carmona et al. 1994). Tritiated versions of each of these three drugs have been used in vitro for autoradiographic imaging studies of cannabinoid receptor density with essentially identical results. Since CB_2-type receptors are nearly absent from normal brain, labeling of brain sections primarily reflects the distribution of CB_1 receptors, even with non-specific radioligands.

Autoradiographic Tracers for Neuronal Activation Studies Ex vivo autoradiography is also commonly used with the glucose analog [^{14}C] 2-deoxyglucose (2-DG) to provide maps of the metabolic demands of neurons (Sokoloff et al. 1977). [^{14}C]2-DG is a substrate for facilitated glucose carriers in the blood–brain barrier

and neuronal cell membranes, and also for hexokinase. Since it cannot proceed further down the glycolytic pathway, its local accumulation as 2-deoxyglucose-6-phosphate reflects local rates of glucose consumption. Radiolabeled 2-DG can be used to assess the metabolic impact of chronic or acute treatments with drugs. Blood flow, another correlate of neuronal activation, can be measured autoradiographically using labeled iodoantipyrine ([^{125}I]IAP and [^{14}C]IAP). This lipophilic, blood flow-dependent ligand has a uniform brain–blood equilibrium partition coefficient throughout the brain, and washes out slowly from the brain allowing enough time for preparation of brain sections.

2.2 Noninvasive Neuroimaging Techniques: PET, SPECT, and MRI

2.2.1 Technique Overview: PET

PET scanners are able to measure the regional and temporal concentrations of positron emitting nuclides in small (4×4×4 mm) volumes of the human body (Phelps 1991). They therefore allow the extension of radioligand binding studies to the living human brain, provided that suitable labeled compounds are available (Gatley 1996). For use in PET, carbon, nitrogen, and oxygen all have positron-emitting isotopes: ^{11}C ($t_{1/2} = 20$ min), ^{13}N ($t_{1/2} = 10$ min) and ^{15}O ($t_{1/2} = 2$ min). Of these, ^{11}C is perhaps most useful because it can be used to label organic compounds, including drugs of abuse, without altering their pharmacokinetics or binding profiles. In addition, many compounds labeled with ^{18}F ($t_{1/2} = 110$ min) are useful PET tracers, because fluorine can often replace –H or –OH with retention of activity.

The high sensitivity of PET scanners allows detection of microgram quantities of radiolabeled molecules in vivo—amounts so small that they do not exert measurable pharmacologic effects and only occupy a small fraction of drug binding sites. Kinetic modeling approaches permit quantitative visualization of the distribution of receptor sites or enzymes within brain or other organs using suitable tracers. Using PET, the behavior of radiolabeled drugs within living systems can be evaluated either without perturbing the system or in the presence of other drugs.

2.2.2 Technique Overview: SPECT

SPECT scanners offer poorer resolution, sensitivity, and quantification than PET scanners but are more widely available because of greater clinical use. Iodine-123 ($t_{1/2} = 13$ h) has the best properties for labeling low molecular weight organic compounds for SPECT while retaining biological activity (Gatley 1993), since an iodine atom is isosteric with a methyl group. Many ^{123}I-labeled radioligands are available. The tracer [^{123}I]iodobenzamide ([^{123}I]IBZM) can be used, like raclopride,

to assess changes in synaptic dopamine (Laruelle 2000). SPECT can also be used to visualize relative perfusion patterns using clinical radiopharmaceuticals labeled with ^{99m}Tc ($t_{1/2} = 6$ h) (Iyo et al. 1997). Cerebral blood flow can be measured quantitatively from washout kinetics of ^{133}Xe, though this method yields poor resolution images with relatively high radiation exposure to the airways.

2.2.3 In Vivo Imaging Radioligands

PET/SPECT Mapping of Cannabinoid Receptors The properties of Δ^9-THC make it unsuitable as a PET CB_1 radioligand. These include extremely high lipophilicity, only moderate affinity for CB_1 receptors, and a structure difficult to label in the time constraints imposed by ^{11}C. In an early study, THC was modified by labeling with ^{18}F in the hydrocarbon side chain. Unfortunately, [^{18}F]THC showed poor brain uptake with a homogeneous distribution in baboon brain (Charalambous et al. 1991), and there was uptake of radioactivity in the skull, suggesting catabolic loss of labeled fluoride ion. It is likely, therefore, that the PET images represented only non-specific uptake of the tracer with a negligible component due to specific binding to cannabinoid receptors. Later radioligand development efforts have largely focused on pyrazole antagonists, as discussed in Sect. 3.1.

PET/SPECT Ligands for Neuronal Activation Studies PET studies are commonly used with the glucose analog [^{18}F]fluorodeoxyglucose (FDG) to provide maps of the metabolic demands of neurons in a manner analogous to the use of 2-DG in autoradiography (Reivich et al. 1977). Like 2-DG, FDG is a substrate for facilitated glucose carriers in the blood–brain barrier. Its accumulation within neurons reflects local rates of glucose consumption and is correlated with neuronal activation. FDG can be used to assess the metabolic impact of chronic or acute treatments with drugs. No SPECT equivalent of the PET tracer FDG is available for brain metabolic studies (Gatley 2003). Blood flow, another correlate of neuronal activation, can be measured with PET using radiolabeled water (Raichle et al. 1983).

2.2.4 Technique Overview: MRI

MRI is another noninvasive strategy that can be used to visualize the effects of drugs of abuse, such as cannabis. Although MRI can be used to answer pharmacologic questions about drugs of abuse, its technique and applications are somewhat different from those of both autoradiography and other in vivo imaging strategies such as PET and SPECT. Rather than detection of radioactivity, MRI involves detection of spin properties of hydrogen nuclei, "protons," which depend on their physical-chemical environments.

Although MRI is not a particularly sensitive technique, it does produce images with excellent spatial resolution (resolution $\ll 1$ mm). Thus anatomical differences

between control subjects and drug abusers can be measured. Furthermore, high-resolution images can be used to more clearly define small brain regions for subsequent analysis using a lower resolution imaging strategy such as PET, or fMRI (see below) in the same individual.

Even though drug concentrations and kinetics can rarely be directly measured within living systems using MRI, because of sensitivity issues, the effect of drugs can be inferred by using MRI to measure the effect of the drug on imaging parameters thought to be correlated with neuronal activity, a technique known as functional MRI (fMRI). The most common fMRI technique is BOLD (blood oxygenation level dependent) scanning, which has excellent time resolution on the order of seconds. The kinetics of changes in neuronal activation caused by drug or by performance of a cognitive or behavioral task can be resolved using MRI with much faster time resolution than using PET, though the spatial resolution of fMRI is similar. Very little cannabinoid research utilizing MRI techniques has yet been reported.

3
Major Topics of Investigations Using Autoradiography

3.1
Measurement of Cannabinoid Receptor Density

CB_1 Receptor Mapping Autoradiographic studies with high-affinity THC analogs both in rat brain tissue (Herkenham et al. 1990) and in postmortem human brain tissue (Thomas et al. 1992; Biegon and Kerman 2001) have demonstrated high concentrations of cannabinoid receptors in the basal ganglia and especially in its outflow nuclei, the globus pallidus, and substantia nigra. High concentrations are also found in hippocampus and cerebellum. The cerebral cortex, especially the cingulate gyrus, also has a fairly high CB_1 receptor density. Some other regions, including most of the brainstem and the thalamus, contain few CB_1 receptors.

Drug Effects on Cannabinoid Receptor Density A number of other studies have assessed the effect of chronic treatments with cannabimimetic drugs on cannabinoid receptor binding. An early study, investigating the mechanism of locomotor tolerance to treatments consisting of 2 weeks of daily i.p. Δ^9-THC or CP 55,940 in rats, reported dose-dependent reductions in binding of radiolabeled CP 55,940. This was attributed to agonist-induced downregulation of CB_1 receptors in striatal brain sections (Oviedo et al. 1993). A later study comparing receptor binding alterations after chronic Δ^9-THC in rats reported that 5 days i.p. administration of Δ^9-THC decreased cannabinoid receptor binding in all brain areas studied, including cerebellum, hippocampus, basal ganglia, limbic nuclei, and cerebral cortex, among others (Romero et al. 1997). CB_1 mRNA levels in these regions were also measured, but did not show reductions in parallel to reductions in receptor binding (Romero et al. 1997). Further research by this group focused on the time-course of receptor down-regulation. Rats were treated with i.p. Δ^9-THC for 1, 3, 7, or

14 days before receptor autoradiography. Downregulation was largely progressive, yet highly variable between brain regions studied. For instance, rapid and robust reductions in [^{3}H]WIN 55,212-2 binding were observed in the dentate gyrus, yet in the basal ganglia reductions in binding were slower in onset and more moderate in degree (Romero et al. 1998); alterations in CB_1 mRNA, where present, occurred after changes in receptor binding. Similar reductions in the binding of [^{3}H]CP 55,940 in rat brain were reported after i.p. Δ^9-THC twice daily for 6 days (Rubino et al. 2000), a regimen that produced maximal reductions. Furthermore, these reductions were accompanied by increases in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activity in the same regions (cerebellum, striatum, globus pallidus, and cortex).

Most autoradiographic studies using postmortem human brain tissue have involved schizophrenic subjects, since a link has been suggested between brain cannabinoid systems and schizophrenia (Arseneault et al. 2004), based, in part, on a high density of CB_1 receptors in brain areas implicated in schizophrenia (Biegon and Kerman 2001). Dean and colleagues studied postmortem tissue from schizophrenic subjects, some of whom had cannabis exposure shortly before death. They associated increases in [^{3}H]CP 55,940 binding in the dorsolateral prefrontal cortex with schizophrenia, but increases in binding in the caudate-putamen with cannabis use (Dean et al. 2001). Another postmortem study of the anterior cingulate cortex revealed a 64% increase in [^{3}H]SR141716A binding in tissue from schizophrenic subjects compared to normal matched controls (Zavitsanou et al. 2004).

Competition for Cannabinoid Receptor Binding Autoradiographic studies in rat brain indicate that anandamide, SR141716A, and CP 55,940 compete for the same cannabinoid receptor, despite the fact that some effects of anandamide are not blocked by the selective CB_1 receptor antagonist SR141716A (Adams et al. 1998). This may partly reflect binding of anandamide to vanilloid receptors (Zygmunt et al. 1999). There are hints that one or more additional central non-vanilloid cannabinoid-type receptors may exist (Calignano et al. 1998; Di Marzo et al. 2000).

3.2 Measurement of Cannabinoid Effects on Neuronal Metabolism

Several studies have examined the effects of Δ^9-THC upon regional cerebral glucose metabolism (rCGM). Biphasic dose-related alterations in glucose utilization in rat have been found. Slight increases in rCGM in limbic and cortical areas but not in diencephalic or brainstem areas were reported after low doses (0.2 mg/kg i.v.) of Δ^9-THC in rats (Margulies and Hammer 1991). Larger doses (2 or 10 mg/kg) decreased rCGM in the cortical and limbic areas. Another paper has shown evidence of increases in rCGM with small doses of Δ^9-THC and decreases with larger doses of Δ^9-THC. Small increases in overall CGM were found using a dose of 1 mg/kg and overall decreases after 5 mg/kg (Brett et al. 2001). Significant reductions in rCGM were seen in rat hippocampal, limbic, sensory, and sensorimotor processing

regions, consistent with the effects of Δ^9-THC on memory, sensory perception, and motor control (Brett et al. 2001). Autoradiography has also been used to examine the time-course of Δ^9-THC effects on rCGM. A single dose (2.5 mg/kg i.p.) of Δ^9-THC resulted in an immediate widespread depression of CGM including limbic areas, and motor and sensory systems. Metabolism returned to baseline in 8 of the 17 structures originally affected after 6 h, and in all but 6 structures after 24 h (Whitlow et al. 2002).

Other researchers have not found biphasic effects of Δ^9-THC on rCGM, although they confirm time- and dose-dependent effects of Δ^9-THC on rCGM in rat brain. Acute administrations of low doses of i.p. Δ^9-THC produced dose-dependent reductions in glucose utilization in rat brain. A dose of 1.0 mg/kg i.p. Δ^9-THC was associated with decreases in rCGM occurring primarily in the limbic and sensory systems, with more widespread reductions occurring after a 2.5 mg/kg dose. If rats were pre-treated with the CB_1 antagonist SR141716A prior to Δ^9-THC administration, no significant reductions in rCGM were observed in 34 of the 38 regions examined (Freedland et al. 2002). Freedland and colleagues reconcile the discrepancy between their findings of decreased rCGM after 1 mg/kg i.p. Δ^9-THC, with the findings of Margulies and others cited above (increased rCGM after 1 mg/kg i.v. Δ^9-THC) by pointing out the different routes of Δ^9-THC administration used in the studies. Intraperitoneal administration of drugs leads to slower onset kinetics compared to intravenous administration, a factor that has been found to have a significant impact on the effects of other drugs of abuse (Porrino 1993). Rats with a tolerance to Δ^9-THC exhibited an altered pattern of glucose metabolism relative to drug-naïve animals. While naïve rats exhibited large global decreases in CGM following a single 10 mg/kg i.p. dose of Δ^9-THC, behaviorally tolerant rats had a more localized reduction of CGM, primarily in regions associated with memory, reward, and stress, such as the hippocampus, nucleus accumbens, mediodorsal thalamus, basolateral amygdala and median raphe (Whitlow et al. 2003).

The effects of synthetic cannabinoid drugs have also been studied using the 2-DG strategy. One study found that the effects of the synthetic cannabinoid agonist WIN 55,212-2 on brain glucose metabolism are largely consistent with the effects of Δ^9-THC. Doses between 0.15–0.30 mg/kg WIN 55,212-2 reduced 2-DG accumulation in the hippocampus and ventrolateral thalamic nucleus of rats (Pontieri et al. 1999). Another study found that acute administration of the CB_1 antagonist SR141716A decreased rCGM in limbic areas thought to be involved in motivated behavior. These findings were accompanied by reduced rates of food-reinforced responding in the same animals. Reductions in both responding and rCGM in limbic systems after SR141617A were more pronounced in animals that had been made tolerant to the behavioral effects of Δ^9-THC (Freedland et al. 2003).

3.3 Measurement of Cannabinoid Effects on Blood Flow

Δ^9-THC and its active metabolite 11-OH-THC both reduce rCBF, as measured using autoradiography with [^{14}C]iodoantipyrine (IAP) . Rats injected with Δ^9-

THC at doses ranging from 0.5 to 16 mg/kg 30 min prior to sacrifice had variably decreased rCBF in 16 brain areas, including the CA1 region of the hippocampus, the frontal and medial prefrontal cortex, the nucleus accumbens, and the claustrum. Other regions, such as the medial septum, caudate, cerebellum, and several other cortex regions, remained unaffected. Similar results were obtained when rats were injected with 11-OH-THC. Most of the regions affected by Δ^9-THC in this study express CB_1, with the notable exceptions of the CA3 region of the hippocampus and the cerebellum, which had no change in cerebral blood flow (Bloom et al. 1997).

Anandamide dose-dependently reduces rCBF in the rat, although it has a short duration of action. At 15 min after 10 mg/kg anandamide, flow was reduced in the amygdala, cingulate, frontal prepyriform, sensorimotor, and claustrocortex. A maximal effect, with 16 additional brain regions involved, was observed after 30 mg/kg anandamide. In most areas, reductions were persistent for 60 min following this larger dose. Anandamide at 3 mg/kg had no effect (Stein et al. 1998).

The limited studies examining the effects of both exogenous and putative endogenous cannabinoids upon regional cerebral blood flow complement studies measuring local brain metabolic activity with 2-DG. They both suggest a strong dose- and time-dependent response to a stimulation of the endocannabinoid system. Together, they support the notion of heterogeneous effects of cannabinoids on brain metabolism.

4
Major Topics of In Vivo Investigations Using PET and SPECT

4.1
Measurement of Cannabinoid Receptor Density

Four types of cannabinoid receptor ligands are currently known: the plant cannabinoids, such as Δ^9-THC and their synthetic relatives such as CP55,940; the endocannabinoids, such as anandamide; the pyrazole ligands, such as SR141716A; and the aminoalkylindole ligands, such as WIN55,2 12-2 (Fig. 1). As mentioned previously, radioligands for the mapping of cannabinoid receptors in vivo are still under development. We have recently reviewed efforts at Brookhaven in collaboration with Dr Alexandros Makriyannis' group (Gatley et al. 2004). Our starting point was to replace the chlorophenyl group of SR141716A with an ^{123}I-iodophenyl group. This produced a tracer (AM251) that was evaluated as a SPECT ligand. Although AM251 gave promising results in mice, and in in vitro autoradiography, it failed to enter the brains of baboons in SPECT experiments (Gatley et al. 1998). A further structural modification of AM251 was performed—insertion of an oxygen into the piperidine ring—yielding AM281, which has lower lipophilicity. In ex vivo experiments in rodents, [^{123}I]AM281 yielded brain autoradiographs similar to those obtained using tritiated ligands in in vitro experiments. Using [^{123}I]AM281 in SPECT experiments, we were able to image CB_1 receptors for the first time in the

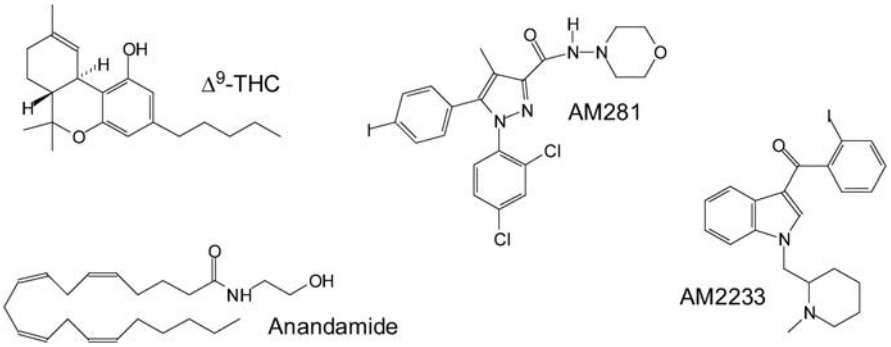

Fig. 1. Representative structures of the different cannabinoid receptor ligand classes: the plant cannabinoid, Δ^9-tetrahydrocannabinol; the endocannabinoid, arachidonoyl ethanolamide (anandamide); the synthetic pyrazole inverse agonist AM281 and the potent aminoalkylindole agonist AM2233. Both AM281 and AM2233 contain an iodine atom that has been labeled with radioiodine for in vitro and in vivo binding experiments

living primate brain (Gatley et al. 1998). The first human brain [^{123}I]AM281 SPECT images of CB_1 receptors have recently been reported by Berding et al. (2004). As anticipated, the extent of specific binding was rather low, and extensive clinical imaging research on CB_1 receptors will probably await development of either a SPECT radioligand with superior properties to [^{123}I]AM281, or, taking advantage of the higher sensitivity of PET cameras, a PET radioligand with at least equivalent brain penetration and receptor affinity to [^{123}I]AM281. Although several candidate PET imaging agents have been synthesized and evaluated biologically by ourselves (Gatley et al. 2004; Gifford et al. 2003) and others (Mathews et al. 2000, 2002; Katoch-Rouse et al. 2003), none has yet been satisfactory.

4.2 Measurement of Cannabinoid Effects on Brain Metabolism

Acute Effects of Δ^9-THC on Brain Glucose Metabolism Two papers have been published utilizing PET to assess the effects of cannabinoids on rCGM in human subjects. (Volkow et al. 1991, 1996). The most consistent observation both in normal controls and habitual marijuana users was an increase in relative metabolic rate in the cerebellum after i.v. Δ^9-THC. This increase was positively correlated both with concentrations of Δ^9-THC in the plasma and with the intensity of self-reported ratings of intoxication. However, the average increase in cerebellar metabolism after Δ^9-THC administration was less in marijuana users than in controls. The FDG/PET studies also demonstrated that marijuana users, but not controls, responded to Δ^9-THC administration with increased metabolic activity in the prefrontal cortex, orbitofrontal cortex, and basal ganglia. Unlike the consistent effects of Δ^9-THC on *relative* metabolic rates, absolute *global* changes were quite variable, as were subjective responses to marijuana or Δ^9-THC. In the studies of Volkow et al. (1991, 1996), Δ^9-THC apparently behaved dissimilarly to acutely administered cocaine, alcohol, morphine, amphetamine, and benzodiazepines (see references

in Gatley and Volkow 1998) in that single acute administrations of Δ^9-THC did not reduce overall metabolic rates. These results are largely consistent with results reported using rCBF as an indicator of neuronal activation (discussed below in Sect. 4.3).

Effects of Chronic Δ^9-THC on Brain Glucose Metabolism Marijuana users had lower baseline cerebellar metabolism than controls (Volkow et al. 1996). This finding, coupled with the finding of increased rCGM after acute exposure to cannabinoids suggests that the decreases in basal cerebellar metabolic rates found in habitual marijuana users may reflect a compensatory response to chronic exposure to the drug. Functions known to be associated with the cerebellum, such as motor coordination, proprioception and learning, are adversely affected both during acute marijuana intoxication and in habitual users of the drug (Varma et al. 1988). The PET scanner used in these investigations lacked sufficient resolution to examine metabolic rates in other brain areas, such as hippocampus, substantia nigra, and caudate nucleus, which contain high concentrations of cannabinoid receptors. However, increased rCGM has not been seen in these areas in rodents using autoradiographic imaging with 2-DG. Studies using modern PET cameras will allow more detailed examination of regional changes in human rCGM induced by acute and chronic Δ^9-THC.

4.3 Measurement of Cannabinoid Effects on Blood Flow

Changes in regional neuronal metabolism are coupled to corresponding regional changes in blood flow. Early ^{133}Xe investigations found bilateral hemispheric increases (right>left) in rCBF 30 min after smoking marijuana compared to smoking placebo in 32 normal human males with a history of exposure to marijuana (Mathew et al. 1992). Increases were greatest in the frontal lobes and were proportional to the reported degree of intoxication. More recent blood flow studies have employed PET and ^{15}O water. An advantage of this tracer is the very short (2 min) physical half-life, which unlike FDG allows repeated measurements in a scanning session. Consequently, ^{15}O water studies can detect brief alterations in rCBF, whereas FDG studies measure accumulation of ^{18}F over a period of about one hour. An ^{15}O water PET study by the Mathew group in 32 normal volunteers found that increases in subject-reported intoxication after i.v. Δ^9-THC doses of 0.15 or 0.25 mg/min over 20 min (total doses of 3 or 5 mg) correlated most markedly with rCBF increases in the right frontal regions, specifically frontal cortex, insula, and cingulate gyrus (Mathew et al. 1997). A subsequent paper showed that the earlier reported increases in rCBF were not present in all 46 subjects studied, and that some subjects showed a decrease in rCBF in the cerebellum that was associated with subject-reported disturbances of time sense (Mathew et al. 1998). A third paper by this group (59 normal subjects) confirmed earlier reports of increased rCBF (right>left hemisphere) and also found increased rCBF not only in frontal lobes, but also in anterior cingulate. The increased rCBF in frontal lobes and anterior

cingulate was associated with subject-reported sensations of depersonalization (Mathew et al. 1999). Most recently, this group has published the time-course of changes in rCBF and behavior after the same doses of Δ^9-THC in 47 normal subjects. This study again confirms earlier findings and additionally reports that blood flow to the cerebellum is increased after the high Δ^9-THC dose (Mathew et al. 2002).

Self-administration of Δ^9-THC by smoking a marijuana cigarette, where the subject controls the dose and the rate of dosing to achieve a desired effect, might be expected to affect rCBF or rCGM differently from experimenter-controlled i.v. Δ^9-THC. PET studies using ^{15}O water, in fact, have shown increases in similar regions (orbitofrontal lobes, insula, and temporal poles), in addition to increased rCBF in the cerebellum, in normal subjects with previous histories of marijuana use ($n = 5$) after smoking marijuana (O'Leary et al. 2000). Rather than measuring blood flow when the subject is in a resting state, this study used a dichotic listening task to measure marijuana effects on task-related changes in rCBF. Large decreases in rCBF were reported during the listening task in temporal lobe areas sensitive to auditory attention effects (O'Leary et al. 2000). A similar PET study from this group using the same technique in 12 experienced subjects assessed the effect of smoking marijuana on task-related rCBF during an auditory attention task (O'Leary et al. 2002). In addition to replication of their earlier findings, this study noted that anterior increases in rCBF occur primarily in paralimbic regions and postulated that these may be related to marijuana's mood-related effects. Also in this study, reduced rCBF was found in several brain regions, including parietal lobe, frontal lobe, and thalamus, which may form part of an attentional network. No rCBF changes were noted in the nucleus accumbens or in any other region thought to be associated with "reward circuitry". Interestingly, brain regions having high densities of cannabinoid receptors, such as basal ganglia and hippocampus, also did not show changes in rCBF (O'Leary et al. 2002). The most recent study from this group assessed the effect of smoked marijuana in 12 occasional and 12 chronic users during counting and finger-tapping tasks accompanied by PET with ^{15}O water. Both counting rate and finger-tapping rate were acutely increased after marijuana smoking and both these effects were correlated with increased blood flow in the cerebellum (O'Leary et al. 2003).

Another group of studies using PET with ^{15}O water have examined the effects of chronic marijuana on rCBF. In contrast to findings after acute marijuana use, chronic users had decreased blood flow to a region of posterior cerebellum after more than 26 h of monitored abstinence from marijuana, compared to control subjects (Block et al. 2000b). Additionally, a later publication using a similar design evaluated effects of chronic marijuana on memory-related blood flow. Decreases in prefrontal cortex, altered lateralization in hippocampus, and increased rCBF in memory-related regions of cerebellum were documented (Block et al. 2002).

The cerebellum is likely to be involved in the psychoactive effects of marijuana. The effects of cannabinoids on rCBF in the cerebellum are consistent with interactions between cannabinoids and the high concentration of CB_1 receptors in this brain area. Both acute marijuana intoxication and habitual use have been shown to affect parameters such as motor coordination, proprioception, and learning,

in which the cerebellum plays a key role (Varma et al. 1988). The lack of significant changes in blood flow in the basal ganglia is somewhat surprising given the facts that firstly, high densities of CB_1 receptors are present in this region, and, secondly, that marijuana serves as a reinforcer in at least a large subset of humans. The endocannabinoid system's modulatory/inhibitory actions on presynaptic neurotransmitter release may complicate the interpretation of regional changes in blood flow after exogenous cannabinoid receptor agonists.

4.4 Other Topics

Stimulant drugs and reinforcers have been shown to be associated with elevated synaptic dopamine that can be monitored in PET studies using the D_2 radioligand [^{11}C]raclopride, whose in vivo binding is sensitive to alterations in extracellular dopamine. We are not aware of any published systematic study of cannabis smoking or Δ^9-THC using this experimental paradigm. However, a study of a single individual was inadvertently conducted with the SPECT tracer [^{123}I]IBZM, whose binding is also sensitive to competition with synaptic dopamine. This study was designed to measure alterations in dopaminergic function in schizophrenics. During the scanning protocol, the subject reported feeling anxious and requested a break. During this break he surreptitiously smoked marijuana. This behavior was revealed the next day during a follow-up mental status exam, which showed significant worsening of psychotic symptoms. Comparison of the subject's scans taken before and immediately after marijuana showed a 20% reduction of D_2 binding potential, attributed to increased synaptic dopaminergic activity (Voruganti et al. 2001). This anecdotal report illustrates some of the challenges of clinical drug abuse research.

5 Major Topics of Investigation using MRI

5.1 Cannabinoid Research Utilizing MRI

Anatomical Studies An early paper using the comparatively primitive technique air encephalography to evaluate neuroanatomical changes in chronic cannabis users reported cerebral atrophy (Campbell et al. 1971), and sparked a debate in the field. Other studies using more advanced techniques such as computerized axial tomography have not substantiated these results (Co et al. 1977; Kuehnle et al. 1977). Two more recent papers utilizing MRI to assess anatomical changes in marijuana using subjects have reported conflicting findings. A study combining both PET with ^{15}O water and structural MRI to evaluate alterations in blood flow as well as structural changes in the brains of 57 subjects found that marijuana users who started using marijuana before the age of 17 had smaller brains than either subjects who began using marijuana later, or control subjects (Wilson et al.

2000). Additionally, the subjects who began using marijuana earlier had smaller gray matter/white matter ratios than the other subjects, and were smaller in overall body size. Finally, the early marijuana users had higher global CBF. In contrast, another MRI research group found no significant structural changes in the brains of 18 frequent users of marijuana (Block et al. 2000a). Although the issue of anatomical alterations in brain after marijuana use remains to be definitively resolved, large studies using combinations of more than one imaging modality are likely to provide the best answers about relationships between the presence or the absence of these changes and the functional impact of marijuana use.

Functional Studies As of March 2004, there have been no peer-reviewed papers utilizing BOLD fMRI to examine functional changes in brain after acute or chronic marijuana. However, a recent meeting abstract hints at the wealth of information that remains to be gathered using this powerful technique. This preliminary study reported that marijuana users had decreased brain activation in cerebellar vermis and dorsal parietal cortex compared to normal control subjects during a visual attention task. Additionally, marijuana users were reported to show exposure-dependent decreases in relative BOLD signal in the cerebellar vermis (Chang et al. 2003). A pilot study of working memory in cannabis smokers, tobaco smokers and non-smoking controls has recently appeared (Jacobsen et al. 2004).

6 Summary

Compared with investigations of other drugs of abuse such as cocaine and opioids, imaging research on brain cannabinoid systems is still in its infancy. Although significant progress has been made using autoradiographic techniques, a great deal of work remains to be done with in vivo imaging. The near future will see clinical studies using fMRI, high-resolution FDG/PET, and PET studies with CB_1 receptor radioligands, and with radioligands for other neuroreceptors. The development of small animal imaging technologies, in the form of microPET cameras and small-bore high-field MRI scanners will allow very tightly controlled studies of cannabinoid drugs and their effects in living animal subjects, which may help resolve the conflicting results that have been reported in the human cannabinoid imaging literature.

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