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REVIEW

Cannabis: pharmacology and toxicology in animals and humans

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Abstract

Cannabis is one of the most widely used drugs throughout the world. The psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces a myriad of pharmacological effects in animals and humans. For many decades, the mechanism of action of cannabinoids, compounds which are structurally similar to Δ^9-THC, was unknown. Tremendous progress has been made recently in characterizing cannabinoid receptors both centrally and peripherally and in studying the role of second messenger systems at the cellular level. Furthermore, an endogenous ligand, anandamide, for the cannabinoid receptor has been identified. Anandamide is a fatty-acid derived compound that possesses pharmacological properties similar to Δ^9-THC. The production of complex behavioral events by cannabinoids is probably mediated by specific cannabinoid receptors and interactions with other neurochemical systems. Cannabis also has great therapeutic potential and has been used for centuries for medicinal purposes. However, cannabinoid-derived drugs on the market today lack specificity and produce many unpleasant side effects, thus limiting therapeutic usefulness. The advent of highly potent analogs and a specific antagonist may make possible the development of compounds that lack undesirable side effects. The advancements in the field of cannabinoid pharmacology should facilitate our understanding of the physiological role of endogenous cannabinoids.

Introduction

Although used for centuries for both medicinal and recreational purposes, no other drug of abuse, as defined by the United States Controlled Substances Act, arouses greater controversy than cannabis. Cannabis use is widespread throughout the world; in fact, it is the most prevalently used drug in many countries. Despite efforts to curtail its use in the United States, cannabis remains one of the most commonly abused drugs, ranking only behind the consumption of alcohol and cigarettes. According to the National Institute on Drug Abuse National Household Survey on Drug Abuse, approximately 59% of adults in the United States between the ages of 26 and 34 have used cannabis in their life-time. Importantly, 2–3% of the population in the United States consume cannabis on a daily basis. Public debate centers upon the possible legalization of cannabis for certain therapeutic uses, such as glaucoma treatment, appetite stimulation in AIDS patients and suppressing nausea resulting from chemotherapy. By the early 1980s extensive research had provided information concerning the identification of cannabinoids in the plant and the physiochemical and biochemical properties of these compounds. Numerous breakthroughs in the past few years have greatly increased our understanding of cannabinoids. The purpose of

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this article is to review the history, chemistry, pharmacology and toxicology of cannabis in both animals and humans. Furthermore, this review will discuss the complex and often ambiguous health consequences and clinical utility of cannabinoids. Recent advances, such as the characterization and cloning of a specific cannabinoid receptor, identification of a second messenger system and isolation of an endogenous ligand, also will be presented to provide insight for the direction of future research for this fascinating drug.

History and prevalence of use

References to the use of the plant *Cannabis sativa*, also known as Indian hemp, date back over 12 000 years (Abel, 1979). The ancient Chinese and Greeks used cannabis to make ropes and clothes. Romans were also aware of the strength of cannabis rope and used it in naval construction. The plant was cultivated for its fiber early in American history at Jamestown, Virginia in 1611 (Grinspoon & Bakalar, 1993). Cannabis has long been used as a medicine in China, India, the Middle East, South Africa and South America. Egyptian, Chinese (2700 BC) and Assyrian (800 BC) sources indicate that it is one of the oldest drugs in history (Mechoulam & Feigenbaum, 1987). The earliest reference to the medicinal properties of cannabis dates back to 2700 BC (Grinspoon & Bakalar, 1993). The Chinese emperor Shen-Nung described cannabis in a book that later became the standard Chinese compendium of medicines. The Chinese used cannabis for treatment of constipation, malaria, rheumatic pains and female disorders. The euphoric properties of cannabis were discovered in India between 2000 and 1400 BC, and cannabis was recommended medicinally for reducing fevers, producing sleep, stimulating the appetite, relieving headaches and curing venereal diseases (Mechoulam & Feigenbaum, 1987).

Cannabis was introduced into western medicine several millennia later following the publication of a treatise in 1839 by W. B. O'Shaughnessy, a 30-year-old Irish physician serving in the British army in India (Snyder, 1971; Lemberger, 1984). He carefully reviewed literature on the uses of cannabis in Indian medicine for over 900 years and found that cannabis was a very safe drug. To further confirm the safety of cannabis, he conducted a series of experiments in animals to determine its effects and dosage limits (Snyder, 1971). He found that cannabis was safe in animals, and even high doses did not kill mice, rats or rabbits. He administered cannabis to patients suffering from seizures, tetanus, rabies and rheumatism and recorded success, although side-effects, such as total catalepsy, sometimes occurred. He noted the anticonvulsive, analgesic, antianxiety and antiemetic properties of the drug. The reports of O'Shaughnessy made cannabis an acceptable form of medicine in England and other European countries (Mechoulam & Feigenbaum, 1987). At the turn of the twentieth century, the medicinal use of cannabis waned in the United States and Europe due to the development of synthetic medicines.

The rising fear of cannabis use in the United States began in the 1920s, and the use of cannabis was abolished in the United States in 1937 with the enaction of the Marijuana Tax Act (Musto, 1987). The Mexican term marijuana refers to cannabis leaves or other crude plant material. Despite legal measures in the United States, cannabis still became a major drug of abuse in the late 1960s, with peak usage occurring in the late 1970s and early 1980s. A United States Bureau of Census report in 1971 indicated that 40% of individuals between 18–25 years of age had experimented with cannabis, and 18% from the same age group currently used the drug. Drug usage data was also obtained in the United States with the National Household Survey on Drug Abuse and the Monitoring the Future Survey, which collected information starting in 1975 from young adults, college age students, twelfth grade students in public and private schools and adding eighth and tenth graders in 1991. According to the Monitoring the Future Survey, 1979 was the peak year of use with 60.4% of twelfth graders having used cannabis in their life-time, and 50.8% of high school seniors in 1979 had used cannabis in the past year (Johnston *et al.*, 1995). In 1978, 37.1% of twelfth graders surveyed had used cannabis within 30 days, and 10.7% used cannabis on a daily basis. Following these peak years cannabis use began a slow, but continuous, decline with the lowest levels of annual use occurring in 1992. In 1992, 21.9% of twelfth graders had used cannabis in the past twelve months, and 1.9% used the drug on a daily basis. The decline in use was linked to an increase in perceived risk and personal disapproval in drug use. Surveys since

1992 indicated significant increases in all use categories (Johnston *et al.*, 1995). From 1992 to 1994, life-time use in twelfth graders increased 5.6%; annual use increased 8.8%; 30-day use increased 7.1%, and daily use increased 1.7%. Although the current levels of cannabis use in the United States are still much lower than the peak periods of use, the recent increases represent an early warning that cannabis use could continue to increase, especially with the high school population. The recent upturn is due to a decline in social disapproval of cannabis and in perceived risk, lower public attention to cannabis and an increase in prodrug messages in popular culture (Hall *et al.*, 1996). The 1994 Monitoring the Future Survey reports that "perceived harmfulness" of cannabis use for all age groups decreased. When asked in 1994 if "great risk" would result if cannabis was "smoked regularly", 65% of twelfth graders reported affirmatively. This response represents a 7.5% decrease from 1993. There was also an increase in participants who found that obtaining cannabis was "fairly or very easy". The increases in cannabis use, decline of perceived harmfulness and decline in social disapproval demonstrate an erosion of the anti-drug attitude in the United States.

Epidemiological evidence is also available from other countries, and these studies are reviewed by Hall *et al.* (1996). In Canada several school studies have shown similar trends to the United States, with a rise in use in the 1970s followed by a decline throughout the 1980s. However, the rates of illicit drug use were lower than in the United States. A national telephone survey reported that 23% of those sampled had ever used cannabis (Hall *et al.*, 1996). Cannabis is the most commonly used illicit drug in Australia. A 1993 national survey of adults demonstrated that one-third had used cannabis (Hall *et al.*, 1996). Large increases in use between 1988 and 1992, especially in males, were reported in the Netherlands from a national survey of students aged 10 to 18.

Although cannabis is used throughout the world, limited survey data is available in other parts of the world. Often this data provides only a crude indication of use. Survey methods are not reported, results are presented in a summary format and the levels of use of teenagers is often under-reported. Nevertheless, these surveys do give an indication of overall levels of use. Limited data is available from Africa (Hall *et al.*, 1996). A survey of 5000 workers reported that a prevalence of 11.5% had used cannabis. In a Moroccan survey from Tangier, two-thirds of 500 students reported had used cannabis. Reported rates of cannabis use in South American countries are lower than in western countries, including the United States, Canada, Europe and Australia. In Brazil, two school-based surveys conducted in 1987 and 1989 demonstrated that 2.9% in 1987 and 3.4% in 1989 had used cannabis. Similar results were found in a 1992 National Household Survey on Drug Abuse in Columbia. Among 18- to 24-year-olds, 1.5% had used cannabis in the past year. In a survey conducted in Athens, Greece, which was based upon the substance use and attitudes sections of similar questionnaires of the World Health Organization and the United Nations, cannabis or hashish was the most frequently used illicit drug (Kokkevi, 1994). The highest life-time rate of cannabis use was found in 25- to 35-year-old males (27.9%). A compilation of limited studies conducted in various hospitals in Lebanon indicated that hashish smoking is common, especially in rural areas where it is almost a habit (Hachem, 1994). One street study found that 142 of 198 participants were hashish users (Hachem, 1994). In Mexico, cannabis has been the most reported drug of initiation in the past three years (Tapia-Conyer *et al.*, 1994). India has a long tradition of cannabis use associated with religious ceremonies (Hall *et al.*, 1996). However, only very limited surveys are available. Surveys conducted in three Northern Indian states between 1989 and 1991 found a life-time prevalence rate of 3% and current use rate of 1%. In Southern India a life-time prevalence rate of 7% has been reported. Higher prevalence rates of 10–27% exist among students. The limited data on cannabis use in African, Asian, Central and South American and Middle Eastern countries suggest that these countries have lower rates of life-time cannabis use than many western countries. Before definite conclusions are drawn, more complete and standardized surveys need to be conducted.

The cannabis plant

The flowering tops and leaves of the plant *Cannabis sativa*, subspecies indica, secrete a resin containing psychoactive compounds called cannabinoids. The highest concentration of cannabinoids in the plant is found in the flowering

tops, followed by the leaves. Small amounts of cannabinoids are found in the stem and roots, and none in the seeds. The cannabinoid content of the plant varies widely depending upon the climate, soil, cultivation and type of plant. The plant is cut, dried and incorporated into cigarettes with or without tobacco. Three types of plant preparations are used, as identified by the Indian names bhang, ganja and charas (Grinspoon & Bakalar, 1993). Bhang is made from dried leaves and tops of uncultivated plants and contains a low resin content. Ganja is obtained from the leaves and tops of cultivated plants and has a higher resin content. The first two preparations are referred to as marijuana. Charas, also known as hashish, is prepared from the resin itself and is 5–10 times stronger than marijuana. Plant products are also chewed, smoked in a waterpipe or eaten in baked goods.

A concern exists that the problem of elevated cannabis use may be compounded by recent increasing concentrations of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive constituent in the plant, found in confiscated cannabis. During the late 1960s, the average level of Δ^9-THC content was 1.5%. The levels steadily increased to the mid-1980s when concentrations had doubled to 3.0–3.5% (ElSohly & Ross, 1994). Seized samples composed primarily of buds and sinsemilla (unfertilized flowering tops from the female *Cannabis* plant) contain much higher concentrations of Δ^9-THC. In fact, samples of cannabis sometimes contain concentrations of Δ^9-THC as high as 20%. Emphasis upon genetic experimentation and cross-breeding in recent years and developments in indoor hydroponic cultivation techniques have led to higher THC content in cannabis plants (Clarke, 1981). These efforts have enhanced THC levels in Dutch hemp ("Netherweed") to concentrations averaging 20% (Hall *et al.*, 1996). One may argue that the elevation in levels of Δ^9-THC has not contributed to cannabis use since there was a decline in use during the time when levels had increased and then stabilized. On the other hand, if highly potent cannabis becomes readily available, the patterns in cannabis use could be affected.

Preclinical studies

Chemistry

The cannabis plant contains over 400 chemical compounds. Approximately 60 of these compounds are cannabinoids and belong to the terpenophenolic chemical class. The term cannabinoid refers to the C21-compounds present in the plant and includes their transformation products and related analogs. The elucidation of the principal psychoactive constituents facilitated the ease of studying the pharmacological and behavioral effects of cannabis' specific constituents. The isolation of cannabinol and cannabidiol in the 1940s provided the general structure of the active principle of cannabis, but neither of these compounds had much psychotomimetic activity (Adams *et al.*, 1940a, 1940b). Mechoulam and his colleagues, in the 1960s, first isolated Δ^9-tetrahydrocannabinol (Δ^9-THC), which was later found to be primarily responsible for the psychoactive properties of the plant (Fig. 1) (Gaoni & Mechoulam, 1964). The pharmacological activity of Δ^9-THC is stereoselective, with the (−)-trans isomer having 6–100 times more potency than the (+)-trans isomer, depending upon the pharmacological test (Dewey *et al.*, 1984). A second psychotomimetic compound was also identified as Δ^8-THC, a positional isomer of Δ^9-THC (Hively *et al.*, 1966). The pharmacological profiles for the two components are similar, with Δ^9-THC possessing somewhat greater potency.

Efforts were undertaken to synthesize and evaluate cannabinoid analogs for the purpose of separating desired pharmacological effects from adverse effects and for the elucidation of the biochemical and molecular mechanisms of cannabinoid action. Initially, due to the lipophilic nature of Δ^9-THC and the central depressant effects, cannabinoids were thought to mediate their actions through disruption of membrane ordering, similar to the mechanism of action of general anesthetics (Paton & Pertwee, 1972; Lawrence & Gill, 1975). Extensive structure–activity relationship studies of cannabinoid analogs revealed strict structural requirements for pharmacological activity and provided early evidence for a specific cannabinoid receptor. Three points of attachment of Δ^9-THC were postulated to interact with a receptor: (1) a free phenolic hydroxyl group; (2) an appropriate substituent at the C9 position, and (3) a lipophilic side chain (Howlett *et al.*, 1988). Although progress in the development of potent cannabinoid agonists has been slower than for other centrally acting agents, potent agonists recently have

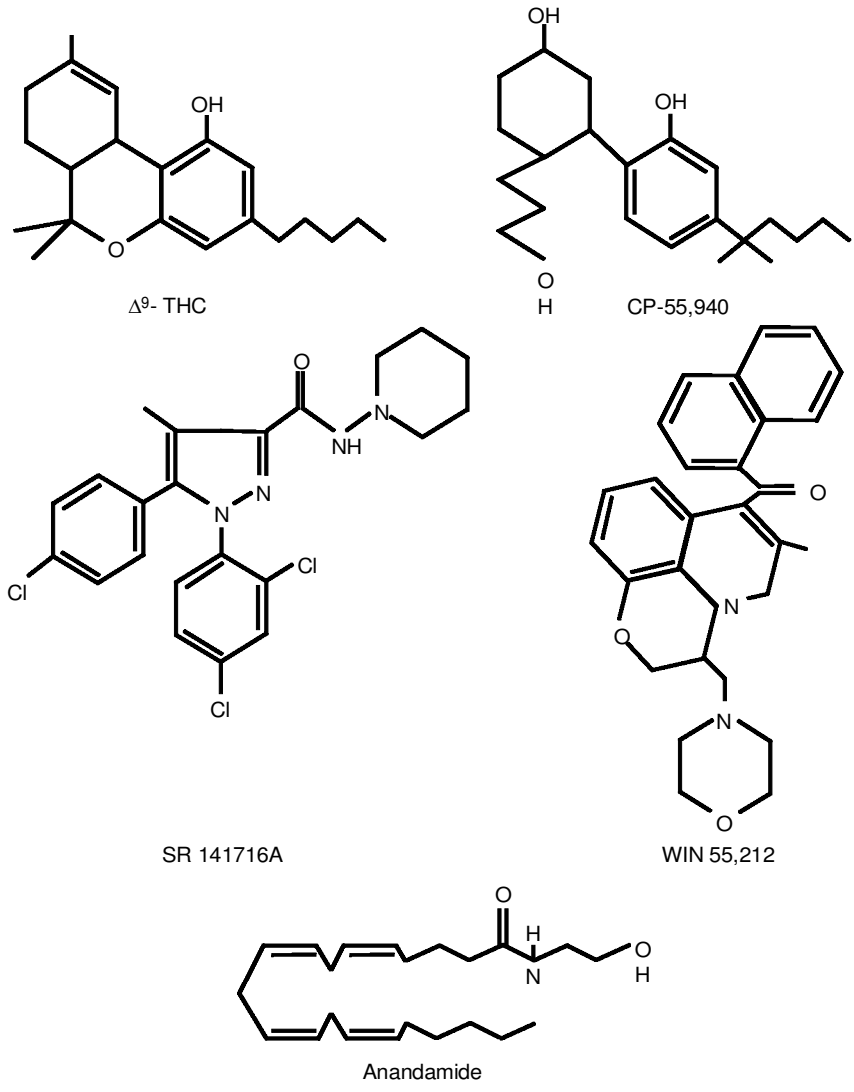

Figure 1. *Structures of Δ^9-THC, CP-55,940, SR 141716A, WIN 55,212 and anandamide.*

emerged and contribute to advancing cannabinoid pharmacology.

Four chemically distinct subclasses of compounds exist with pharmacological and behavioral similarities to Δ^9-THC, including compounds possessing three rings, such as Δ^9-THC, bicyclic compounds, aminoalkylindoles and anandamides. Potent compounds have resulted from numerous structural alterations made to the basic template of Δ^9-THC. Substitution of the pentyl group with a dimethylheptyl side chain and hydroxylation at carbon 11 of Δ^8-THC resulted in 11-OH-Δ^8-THC-DMH, a compound several hundred times more potent than Δ^8-THC in various behavioral assays (Mechoulam *et al.*, 1988; Little *et al.*, 1989). A corresponding pharmacologically potent derivative of Δ^9-THC, 11-OH-Δ^9-THC-DMH, was also developed (Razdan, 1987; Martin *et al.*, 1991). In addition, the use of highly pure enantiomers of 11-OH-Δ^8-THC-DMH established firmly that cannabinoids exhibit enantioselectivity (Mechoulam *et al.*, 1988). The existence of enantiomers reinforced the notion

that cannabinoids act through a specific receptor. Synthesis of the dimethylheptyl derivatives was based upon the three-point attachment theory and the necessity of an intact dibenzopyran ring system. While attempting to develop a unique analgesic, a group at Pfizer prepared novel bicyclic cannabinoid analogs with pharmacological profiles similar to Δ^9-THC (Melvin *et al.*, 1984). CP-55,940 (Fig. 1), the most widely used compound in the series, possessed potency 4–25 times greater than Δ^9-THC, depending upon the pharmacological assay. Due to the divergence in structure, great attention was placed upon proving that the bicyclic analogs were definitely THC-like. Evidence that CP-55,940 is a cannabinoid emerged from studies showing that CP-55,940 and Δ^9-THC cross-generalized in rats and monkeys, and cross tolerance developed between the two compounds (Gold *et al.*, 1992; Pertwee *et al.*, 1993).

Although the bicyclic analogs contained unique characteristics, they still retained most of the structural characteristics of Δ^9-THC. A third class of compounds structurally differed greatly from other classical and bicyclic cannabinoids. Pravadoline, a non-steroidal anti-inflammatory agent, had analgesic properties, but did not interact with the opioid system (Ward *et al.*, 1990). Interestingly, pravadoline produced antinociception through a dual mechanism of action of either inhibiting cyclo-oxygenase or a noncyclo-oxygenase related antinociceptive mechanism. The search for the compound's mechanism of action led to the development of aminoalkylindoles, such as WIN 55,212 (Fig. 1) (Ward *et al.*, 1991). These compounds, although structurally quite distinct from other cannabinoids acted in a similar manner to Δ^9-THC in mice and rats (Compton *et al.*, 1992a). The discovery of anandamide (arachidonylethanolamide) as a proposed endogenous cannabinoid ligand added yet another novel class to the pharmacophore of compounds that produce effects similar to Δ^9-THC (Fig, 1) (Devane *et al.*, 1992). Confirming the similarity of anandamide with Δ^9-THC requires future testing, particularly in humans. The emergence of four chemically distinct classes of "cannabinoids" will provide probes for studying the diverse actions of cannabinoids, and these probes should facilitate the separation of the psychoactive properties from the pharmacological effects.

Animal models and pharmacology

The purpose of evaluating cannabinoids in animals is to establish a parallel relationship between animals and humans and to extrapolate the animal effects to humans. The development of a number of animal models in the mouse, rat, dog, rabbit and monkey have allowed researchers to predict the psychoactivity of novel compounds. Extensive reviews of these results are found elsewhere (Dewey, 1986; Razdan, 1986). Pharmacological effects have been measured with models such as dog ataxia (Loewe, 1947), the THC-seizure susceptible rabbit (Consroe *et al.*, 1982), monkey overt behavior (Grunfeld & Edery, 1969; Edery *et al.*, 1971; Edery *et al.*, 1972), drug discrimination (Balster & Prescott, 1992) and a mouse behavioral battery consisting of spontaneous locomotor activity, hypothermia, immobility (catalepsy) and antinociception (Martin, 1985). Although cannabinoids have direct cellular actions on peripheral tissues, most of the behavioral and pharmacological effects studied by researchers appear to involve the central nervous system (Dewey, 1986). The high lipophilicity of cannabinoids allows passage across the blood brain-barrier.

Cannabinoids generally cause a reduction in spontaneous locomotor activity (Little *et al.*, 1988) and a decrease in response rates with different reinforcement schedules (Carney *et al.*, 1979; Zuardi & Karniol, 1983). Cannabinoids produce a unique syndrome of effects on the behavior of a wide variety of animal species. These behavioral effects are characterized at low doses as a unique mixture of depressant and stimulatory effects and at higher doses as predominantly CNS depression (Dewey, 1986). The depressant effects of psychotomimetic cannabinoids differ from other CNS depressants. Δ^9-THC and other psychoactive cannabinoids in mice produce a "popcorn" effect. Groups of mice in an apparently sedate state will jump (hyperreflexia) in response to auditory or tactile stimuli. As animals fall onto other animals, they resemble corn popping in a popcorn machine. This state of hyper-reflexia is observed during the depressant stage at higher doses (Dewey, 1986).

With the drug discrimination model, animals use internal cues to discriminate between the subjective effects of different drug classes. In this paradigm, rats, pigeons or non-human primates are trained to make different responses for re-

inforcement contingent upon administration of either the training drug or vehicle (Weissman, 1978; Järbe & Hiltunen, 1987; Gold *et al.*, 1992). After successful discrimination training, other drugs may be administered to see if they produce similar stimulus characteristics as the training drug. A correlation exists between drugs that generalize to Δ^9-THC and bind to the cannabinoid receptors. The bicyclic compounds (Gold *et al.*, 1992) and the aminoalkylindoles (Compton *et al.*, 1992b) substitute for Δ^9-THC, whereas drugs from other classes do not (Balster & Prescott, 1992). Ultimately, the best model for evaluating a drug's reinforcing effects and predicting abuse liability is a drug self-administration paradigm. Animals do not readily self-administer Δ^9-THC (Harris *et al.*, 1974), and CP-55,940 does not maintain intravenous self-administration with a fixed-interval schedule in rhesus monkeys (Mansbach *et al.*, 1994). The lack of reinforcing effects of cannabis is consistent with the limited dependence properties in humans. On the other hand, it is possible that animals will self-administer cannabinoids once appropriate models are discovered.

Cannabinoids also impair learning and memory in rodents (Carlini *et al.*, 1970) and non-human primates (Ferraro & Grilly, 1973). In rats, the delayed match-to-sample task (DMTS) (Heyser *et al.*, 1993), Lashley III maze (Carlini *et al.*, 1970) and the eight arm radial-maze (Nakamura *et al.*, 1991) were used to measure memory disruption by cannabinoids. State-dependent learning (SDL) studies have been used to examine the influences of drugs upon the process of conditioning, or memory formation and retrieval. SDL occurs when an association learned in one condition is more easily retrieved in that same condition. This paradigm has been useful for determining some of the disruptive effects of Δ^9-THC on memory and performance. The effects of Δ^9-THC upon SDL have been reported in tasks involving avoidance learning and conditioned suppression (Järbe & Mathis, 1992). Tolerance does develop to the disruptive and subjective drug-state effects of Δ^9-THC (Järbe, 1978; Järbe & Mathis, 1992).

The high numbers of cannabinoid receptors in the hippocampus (discussed further in the following section) may mediate the disruption in cognition (Herkenham *et al.*, 1991c; Jansen *et al.*, 1992; Thomas, Wei & Martin, 1992). Intrahippocampal administration of CP-55,940 produced a dose-dependent increase in the number of errors in the eight arm radial-maze test without elicitation of other pharmacological effects (Margulies & Hammer, 1991). Another study showed that the disruption in the DMTS task induced by acute administration of Δ^9-THC was similar to that produced by damage to the hippocampus (Heyser *et al.*, 1993). This disruption was associated with a specific decrease in hippocampal cell discharge only during the encoding phase of the task; the effects were reversible within 24 hours of dosing. A number of other studies have examined the effects of cannabinoids on hippocampal ultrastructure, and are reviewed by Solowij (1996a). While Δ^9-THC, CP-55,940 and WIN 55,212-2 all impaired working memory in rats, anandamide failed to do so in either the eight arm radial-maze or the delayed non-match-to-sample task (Crawley *et al.*, 1993). Lichtman *et al.* (1995) also found that CP-55,940, Δ^9-THC and WIN 55,212-2 administered systemically impaired spatial memory in rats, as assessed by the eight arm radial-maze, and retarded completion time; neither anandamide nor cannabidiol affected memory. Intrahippocampal administration of CP-55,940 impaired memory, but did not inhibit completion time. The intrahippocampal effects of CP-55,940 appeared specific to cognition since no other pharmacological effects were produced (Lichtman *et al.*, 1995). The inability of anandamide to disrupt memory in rats illustrates a possible difference between the endogenous ligand and other cannabinoids and underscores the importance of further comparisons (Crawley *et al.*, 1993; Lichtman *et al.*, 1995). In a series of chronic studies, rhesus monkeys were trained for 1 year to perform operant tasks before 1 year of chronic cannabis administration (Slikker *et al.*, 1992). Task performance was impaired for over a week after cessation of use, but performance returned to baseline levels 3 weeks after cessation. The effects of chronic exposure were reversible with no apparent long-term behavioral effects.

Mechanism of action

Cannabinoids produce a myriad of pharmacological and behavioral effects which most probably involve numerous neural substrates that traverse the entire brain. The complexity of the pharmacological effects produced by cannabinoids is reflected in the above discussion on

animal models. The most probable candidate for mediating the central effects of cannabinoids is a receptor mechanism. Discerning the mechanism of action for cannabinoids transpired over several decades, and many difficulties encumbered the progress. Enantioselectivity provided initial evidence of receptor involvement. In several behavioral assays, the naturally occurring (−)-enantiomer of trans-Δ^9-THC exhibited 5–100 times more potency than the (+)-enantiomer (Edery *et al.*, 1971; Jones *et al.*, 1974; Martin *et al.*, 1981; Dewey *et al.*, 1984). Subsequently, several synthetic cannabinoid enantiomers have been shown to display pharmacological enantioselectivity (Little *et al.*, 1988, 1989; Mechoulam *et al.*, 1990; Compton *et al.*, 1992b). Often huge differences in potency occur between the enantiomeric pairs. Such high degrees of enantioselectivity indicate a very specific mechanism of action, such as that involving a receptor. The lack of an appropriate radiolabeled ligand greatly hindered proving that cannabinoids exerted their interactions through a specific central receptor. Early attempts to identify a receptor in crude rat brain membranes by a ligand binding assay using ^{3}H-Δ^8-THC failed (Harris, Carchman & Martin, 1978). Saturable binding did not result, and only 10% displaceable binding could be achieved.

The synthesis and radiolabeling of potent bicyclic cannabinoids, such as CP-55,940 (Melvin & Johnson, 1987), allowed identification of a receptor in rat brain membranes (Devane *et al.*, 1988). Analysis of the data revealed a single binding site that possessed saturable and reversible binding. Other labeled cannabinoids, such as the dimethylheptyl (DMH) derivative of ^{3}H-11-OH-Δ^9-THC (Thomas *et al.*, 1992) and ^{3}H-WIN 55,212-2 (Haycock *et al.*, 1991; Compton *et al.*, 1992a), also bind to this receptor. This receptor displays selectivity for cannabinoids, as other centrally acting compounds do not compete for cannabinoid binding (Howlett *et al.*, 1992). Pharmacological potency of cannabinoids correlates well with their affinity for the cannabinoid binding site (Compton *et al.*, 1993). In addition, binding affinities correlated with *in vivo* potency in the rat drug discrimination model and psychotomimetic activity in humans. These findings suggest that this receptor mediates most of the central cannabinoid effects across different species.

According to autoradiographic studies, the distribution of the cannabinoid receptor is heterogeneous in several mammalian species, conserved and neuronally located (Herkenham *et al.*, 1990, 1991b, 1991c). The densest binding occurs in the basal ganglia (substantia nigra pars reticulata, globus pallidus, entropeduncular nucleus and lateral caudate putamen) and the molecular layer of the cerebellum. Binding in these regions may explain cannabinoid interference with movement. Intermediate levels of binding were found in the CA pyramidal cell layers of the hippocampus, the dentate gyrus and layers I and VI of the cortex. Δ^9-THC disrupts short-term memory in humans (Chait & Pierri, 1992). Thus, cannabinoid effects on memory and cognition are consistent with receptor localization in the hippocampus and cortex. The hippocampus stores memory and codes sensory information. The presence of cannabinoid receptors in regions associated with mediating brain reward (ventromeidal striatum and nucleus accumbens) suggests an association with dopamine neurons. Sparse levels were detected in the brain stem, hypothalamus, corpus callosum and the deep cerebellum nuclei. Low levels of receptors in brain stem areas controlling cardiovascular and respiratory functions is also consistent with the lack of lethality of cannabis. Other ligands, such as ^{3}H-WIN 55,212 (Jansen *et al.*, 1992) and ^{3}H-11-OH-Δ^9-THC-DMH (Thomas *et al.*, 1992), generated similar localization patterns. Binding has also been found in the peripheral B lymphocyte-enriched areas including the marginal zone of the spleen, nodular corona of Peyer's patches and the cortex of the lymph nodes (Lynn & Herkenham, 1994).

Prior to the characterization of a receptor, data demonstrated that cannabinoids inhibited adenylyl cyclase by probable interaction with an inhibitory G protein (G_i) (Howlett & Fleming, 1984). Researchers proposed that a cannabinoid receptor was linked to a G_i protein which, when activated, inhibited the activity of adenylyl cyclase. Adenylyl cyclase cannot then catalyze the conversion of ATP to the second messenger cyclic AMP (cAMP). The inhibition of adenylyl cyclase by cannabinoids took place in neuroblastoma cell membranes, rat brain slice membranes and cultured cerebellar neurons (Howlett, Qualy & Khachatrian, 1986; Bidaut-Russell, Devane & Howlett, 1990; Pacheco, Ward & Childers, 1993). While extensive *in vitro* evidence exists for a cannabinoid receptor/adenylyl cyclase interac-

tion, determining a pharmacological effect produced by adenylyl cyclase inhibition is difficult. Recently, Welch, Thomas & Patrick (1995) demonstrated that pertussis toxin blocked the antinociceptive properties of cannabinoids in mice. Pertussis toxin prevents G_i proteins from interacting with receptors. This work suggests that the analgesic properties of cannabinoids might be due to cannabinoid receptor activation of a G_i protein. Furthermore forskolin, which stimulates adenylyl cyclase, thereby producing increased levels of cAMP, and chloro-cAMP, a stable analog of cAMP, decreased cannabinoid-induced antinociception (Welch *et al.*, 1995). Thus, both preventing G_i proteins from interacting with cannabinoid receptors and increasing the levels of cAMP interfered with the production of antinociception. These data suggest involvement of adenylyl cyclase in the antinociception of cannabinoids.

Definitive evidence for a specific cannabinoid receptor became apparent with the cloning of a cannabinoid receptor (Matsuda *et al.*, 1990). A clone isolated from a rat brain library had homology with other receptors that interacted with G proteins in the cell membrane. However, none of the traditional agonists of G proteins bound to this receptor clone. An identification breakthrough occurred with the discovery that the mRNA distribution of the receptor clone paralleled that of the cannabinoid receptor. Confirmation of the identity of the clone occurred when cells transfected with this clone inhibited adenylyl cyclase upon exposure to CP-55,940 and Δ^9-THC. Adenylyl cyclase in non-transfected cells did not respond to cannabinoids. The human cannabinoid receptor was subsequently cloned and found to have almost identical homology to the rat receptor (Gérard *et al.*, 1991). The cannabinoid receptor, abbreviated as CB_1, belongs to a G protein coupled receptor subfamily which includes the adrenocorticotropin and melanotropin receptors (Mountjoy *et al.*, 1992). Recently, a distinct peripheral cannabinoid receptor, designated CB_2, was identified in macrophages in the marginal zone of the spleen (Munro, Thomas & Abu-Shaar, 1993). Although CB_1 and CB_2 share only approximately 40% homology, Δ^9-THC and CP-55,940 demonstrate similar binding affinity for both receptor subtypes. The cloning of a peripheral receptor is consistent with previous data showing cannabinoid binding to mouse spleen cells (Kaminski *et al.*, 1992) and to the rat immune system (Lynn & Herkenham, 1994). CB_1 RNA transcripts have been identified in mouse spleen cells (Kaminski *et al.*, 1992) and human peripheral blood lymphocytes (Bouaboula *et al.*, 1993); CB_2 RNA transcripts are expressed in the rat spleen (Munro *et al.*, 1993). The role of this receptor in the spleen remains unknown. The discovery of a second receptor raises the possibility that other receptors with unique functional roles may exist.

Cannabinoids also produce effects through second messenger systems other than adenylyl cyclase. Initial evidence implicating calcium came from a study in which Δ^9-THC inhibited calcium uptake following depolarization in mouse brain synaptosomes (Harris & Stokes, 1982). Electrophysiological studies showed that cannabinoids inhibited an omega conotoxin-sensitive, high voltage-activated N-type calcium channel (Caulfield & Brown, 1992; Mackie & Hille, 1992). The inhibition of calcium was pertussis toxin-sensitive and stereospecific, suggesting a receptor-mediated process. In contrast, other data demonstrated that calcium influx in transfected cells was not mediated by receptors (Felder *et al.*, 1992). Cannabinoids have also been reported to mediate an enhancement of A-type potassium channels in cultured hippocampal neurons through the cannabinoid receptor (Deadwyler *et al.*, 1993). In addition, an inwardly rectifying potassium channel co-expressed with the neuronal cannabinoid receptor in *Xenopus* oocytes was activated by WIN 55,212-2 (Henry & Chavkin, 1995). The precise role of calcium or potassium in the physiological actions of cannabinoids remains unknown.

Other systems have been proposed for the signal transduction of cannabinoid receptor activation, although the evidence is not as compelling. Some data suggest that cannabinoids might activate the inositol phospholipid pathway. In this signaling pathway, a receptor activates a G protein (tentatively called G_p) that in turn activates phospholipase C. This enzyme cleaves PIP_2 (phosphatidylinositol-bisphosphate) into inositol triphosphate (IP_3) and diacylglycerol. Diacylglycerol activates protein kinase C, and IP_3 triggers calcium release from cellular compartments. One study presented evidence that Δ^9-THC decreased the formation of *myo*-inositol triphosphate in pancreatic island cells (Chaudry *et al.*, 1988). This evidence implies that cannabinoids

bind to a receptor that is linked to the inositol phospholipid pathway. Another study demonstrated that protein kinase C distribution did not co-localize with cannabinoid binding (Herkenham *et al.*, 1991a). If cannabinoids did bind to receptors that activated this pathway, one would assume that cannabinoid binding would co-localize with components, such as protein kinase C, of the inositol phospholipid system. Other researchers showed that cannabinoids also stimulated the release of arachidonic acid and phospholipid turnover (Felder *et al.*, 1992). This effect lacked enantioselectivity, and high concentrations were used. Thus, these investigators ruled against receptor involvement (Felder *et al.*, 1992).

The discovery of a receptor raised the question about the possible existence of an endogenous ligand and a separate cannabinoid neurochemical system. Due to the high lipophilicity of cannabinoids, Devane *et al.* (1992) searched for a compound in lipid extracts from porcine brain. They isolated anandamide, which competed for cannabinoid receptor binding and, like Δ^9-THC, inhibited electrically stimulated contractions in the murine vas deferens (Devane *et al.*, 1992). Anandamide produced similar pharmacological effects to Δ^9-THC, such as antinociception, catalepsy, hypomotility and hypothermia (Fride & Mechoulam, 1993), and anandamide inhibited adenylyl cyclase (Felder *et al.*, 1993) and N-type calcium channels (Mackie, Devane & Hille, 1993). Anandamide, a fatty acid derivative, binds both to the cannabinoid receptor of the rat brain (Devane *et al.*, 1992) and to murine Ltk-cells transfected with the human cannabinoid receptor (Felder *et al.*, 1993). A comparison between anandamide and Δ^9-THC revealed that anandamide was 4–20-fold less potent and had a shorter duration of action than Δ^9-THC (Smith *et al.*, 1994). Both anandamide and Δ^9-THC affected the hypothalamo–pituitary–adrenal axis in a similar manner (Weidenfeld, Feldman & Mechoulam, 1994). Intracerebroventricular administration of anandamide decreased CRF-41 levels in the median eminence and increased serum ACTH and corticosterone levels.

One of the qualifications of a neurochemical system is the existence of a path for synthesis and degradation of a ligand. Deutsch & Chin (1993) showed that anandamide was rapidly taken into neuroblastoma and glioma cells and degraded by an amidase, which resides in the membrane fractions. Degradation also occurred in tissues from the brain, kidney, liver and lung (Deutsch & Chin, 1993). Synthesis was achieved by incubating arachidonate with ethanolamide. The enzyme inhibitor phenymethylsulfonyl fluoride (PMSF) prevented degradation, but not synthesis, of anandamide. Other investigators found that PMSF did inhibit anandamide synthesis in bovine brain (Devane & Axelrod, 1994). Interestingly, lower levels of synthetic anandamide activity were found in the cerebellum, which contains a very high density of receptors. The enzyme involved in the synthesis reaction of anandamide functions through a CoA- and an ATP-independent pathway (Kruszka & Gross, 1994). Evidence for an alternative pathway for anandamide biosynthesis exists. Anandamide formation also occurs through a phosphodiesterase-mediated cleavage of a novel phospholipid precursor, *N*-arachidonoyl-phosphatidylethanolamine (Di Marzo *et al.*, 1994).

The establishment of a cannabinoid receptor and an endogenous ligand with biosynthetic and degradative pathways suggests the possible presence of a distinct neurochemical system. Anandamide may represent one member of a family of endogenous compounds. Two other compounds, homo-γ-linolenylethanolamide and docosatetraenylethanolamide, isolated from bovine brain also competed for cannabinoid receptor binding (Hanus *et al.*, 1993; Mechoulam *et al.*, 1994). Future research must answer numerous questions in order to advance our understanding of the physiology and neurochemistry in the brain. Why does such a system exist? What is its physiological role? What would be the physical manifestations of an imbalance in this system?

The recent discovery of a cannabinoid antagonist should help researchers solve these questions (Rinaldi-Carmona *et al.*, 1994). The antagonist, SR 141716A (Fig. 1) has high affinity for the CB_1 receptor and antagonizes cannabinoid-induced inhibition of adenylyl cyclase and smooth muscle contractions (Rinaldi-Carmona *et al.*, 1994). It also antagonizes cannabinoid drug discrimination in rats (Wiley *et al.*, 1995).

Based upon the discoveries made during the past decade, one can postulate that a "cannabinoid" neurochemical system does exist. The function of this system and its interaction with other neurochemical systems remains unclear. It is well known that cannabinoids exert

many of their actions by influencing several traditional neurotransmitter systems, as presented in other reviews (Dewey, 1986; Pertwee, 1988, 1992). The results from numerous studies suggest that several neurotransmitters and neuromodulators have a role in the neuropharmacology of cannabinoids. These substances include acetylcholine (ACh), dopamine (DA), γ-aminobutyric acid (GABA), histamine, 5-hydroxytryptamine (5-HT), norepinephrine (NE), opioid peptides and prostaglandins (PGEs). The basis for some of the effects of cannabinoids are studied by determining the interaction between cannabinoids and drugs that bind to other receptor types or drugs that alter the synthesis, storage, release or metabolism of transmitters and modulators (Pertwee, 1992). Cannabinoids have been shown to enhance the formation of NE, DA and 5-HT. Cannabinoids also stimulated the release of DA from rat corpus striatum, nucleus accumbens and medial prefrontal cortex. GABA turnover is enhanced by cannabinoids. The most commonly studied effects of cannabinoids include hypothermia, antinociception and changes in locomotor activity. Results from drug interaction studies for catalepsy and depression of spontaneous locomotor activity suggest that these effects are mediated by ACh acting at muscarinic and nicotinic receptors, GABA, acting at $GABA_A$ and $GABA_B$ receptors, and PGEs. The extrapyramidal system probably plays a role in catalepsy, since intrapallidal administration of 11-OH-Δ^8-THC produced catalepsy (Pertwee & Wickens, 1991). Catalepsy results from interaction of Δ^9-THC with neurotransmitter systems in the basal ganglia (Gough & Olley, 1977, 1978). Hypothermia in rats and mice is mediated by DA, NE, 5-HT, GABA, histamine and opioid peptides. There is also evidence that alteration in thermoregulation occurs by the hypothalamus (Fitton & Pertwee, 1982) and brain stem activity (Hosko, Schmeling & Hardman, 1981). Possibly, enhanced serotonergic transmission (Davies & Graham, 1980) and modulated autonomic activity (Rosenkrantz, 1983) produce hypothermia. Results from hypothermia studies are often inconsistent, thus definite conclusions cannot be drawn about the neuronal pathway involved in cannabinoid-induced antinociception (Pertwee, 1992). Several endogenous compounds serve to inhibit nociception (NE, 5-HT, ACh, GABA, opioid peptides, PGE_1 and PGD_2), and some of these compounds interact with cannabinoids to produce antinociception. Data support the involvement of PGE_1, and some experiments support the involvement of catecholamines, 5-HT and opioid peptides. Interpretation of the actions of cannabinoids on neurotransmitter systems is often difficult since evidence exists that cannabinoids both inhibit and stimulate neuronal uptake. Relatively few studies have examined the long-term exposure of cannabinoids on brain neurotransmitter and neuromodulator levels. As reviewed by Solowij (1996a), recent evidence suggests that few, if any, irreversible effects on brain chemistry exist due to Δ^9-THC administration.

Animal tolerance and dependence

Tolerance develops to the pharmacological effects of cannabinoids in a variety of animal species, including pigeons, rodents, dogs, monkeys and rabbits. Several review articles discuss the issues of tolerance and dependence (McMillan, Dewey & Harris, 1971; Kaymakcalan, 1973; Wikler, 1976; Compton *et al.*, 1990). Tolerance has occurred to antinociception (Martin, 1985), anticonvulsant activity (Colasanti, Lindamood & Craig, 1982), catalepsy (Pertwee, 1974), depression of locomotor activity (Karler, Calder & Turkanis, 1984), hypothermia (Thompson *et al.*, 1974), hypotension (Birmingham, 1973), corticosteroid release (Miczek & Dihit, 1980), ataxia in dogs (Martin *et al.*, 1976) and schedule-controlled behavior (McMillan *et al.*, 1970). Tolerance does not develop to all cannabinoid effects, such as ACTH secretion (Dewey, Peng & Harris, 1970). Often the levels of tolerance are markedly high with reported instances of 100-fold development. Other psychoactive cannabinoids, such as Δ^8-THC, the 11-hydroxy metabolites, nantradol and nabilone also produce tolerance (Kosersky, McMillan & Harris, 1974; Watanabe, Yamamoto & Yoshimura, 1983). Interestingly, tolerance has also been demonstrated in cultured cells. Tolerance developed to cannabinoid-induced stimulation of prostaglandin E_2 production and aracidonate release (Burstein, Hunter & Renzulli, 1985) and to cannabinoid-inhibition of adenylyl cyclase activity (Dill & Howlett, 1988).

The precise mechanism for the development of tolerance remains unknown. Tolerance to drugs usually occurs by two main methods: changes in pharmacokinetics or pharmaco-

dynamics. Several lines of evidence indicate that pharmacokinetics (absorption, distribution, metabolism and excretion) probably plays a minor role in tolerance production (Dewey *et al.*, 1973; Siemens & Kalant, 1974; Martin *et al.*, 1976). Thus, a pharmacodynamic event, such as receptor downregulation, receptor conformational change and receptor internalization, more than likely attributes to tolerance development. These three events result in decreased receptor–ligand interaction. Changes at the cannabinoid receptor level following exposure to cannabinoids for a long period of time could result in conformational changes in the receptor which would produce an altered receptor structure to which the ligand could not bind. Another possible pharmacodynamic event is receptor internalization. When receptor internalization occurs, receptors on the cell membrane are removed into the cytoplasm, where they are either degraded or recycled. The number of receptors at the cell surface is decreased; therefore, binding to the receptor is decreased. Several groups have demonstrated cannabinoid receptor downregulation in cannabinoid-tolerant animals (Oviedo, Glowa & Herkenham, 1993; Rodríguez de Fonseca *et al.*, 1994). Receptor downregulation occurs when the number of receptors made by the cells is reduced. Oviedo *et al.* (1993) presented data suggesting that cannabinoid tolerance was due in part to agonist-induced receptor downregulation. Altered binding in animals treated acutely with Δ^9-THC or CP-55,940 resulted from changes in affinity; in chronically treated animals, changes in binding were attributed to a lowering of binding capacity. Rodríguez de Fonseca *et al.* (1994) found that behavioral tolerance developed in rats chronically treated with Δ^9-THC. This tolerance was accompanied with decreases in binding in the striatum and limbic forebrain. In a recent study, cannabinoid binding actually increased in brain areas, such as the cerebellum and hippocampus, after acute or chronic exposure to either anandamide or Δ^9-THC (Romero *et al.*, 1995). No changes were detected in the limbic forebrain or the medial basal hypothalamus, and after chronic exposure receptors were downregulated in the striatum. Interestingly, another study noted that cannabinoid receptor properties were not irreversibly altered by chronic exposure in either rat brain 60 days following 90 days of administration of Δ^9-THC or in monkey brain 7 months after 1 year of exposure to cannabis smoke (Westlake *et al.*, 1991). Receptor down regulation could either result from or cause alterations in gene transcription. Another study found that although a 27-fold behavioral tolerance to Δ^9-THC was observed, neither receptor binding nor mRNA levels in whole brain changed (Abood *et al.*, 1993). Fan *et al.* (1996) have demonstrated that an increase in cannabinoid receptor mRNA accompanies the downregulation of the receptor in the cerebellum of tolerant mice, but cause and effect have not been established.

In light of the fact that most drugs which are used for recreational purposes produce some form of physiological dependence and the fact that development of tolerance frequently occurs in conjunction with dependence, it would seem likely that physical dependence would also develop following chronic exposure to cannabinoids. One of the most common methods for demonstrating dependence, particularly for drugs which do not have a long duration of action, is to abruptly terminate chronic administration of the agent and observe the ensuing behavioral sequelae. Efforts to conduct abrupt withdrawal studies with cannabinoids have produced conflicting results. McMillan *et al.* (1971) failed to detect withdrawal symptoms upon termination of chronic administration of cannabinoids. A few reports have noted that abrupt cessation of cannabinoids produce certain behavioral changes. These alterations include increased grooming, motor activity (Kaymakcalan, Ayhan & Tulunay, 1977), aggression (Beardsley, Balster & Harris, 1986) and susceptibility to electroshock-induced convulsions (Karler *et al.*, 1984). However, re-administration of a cannabinoid did not reverse these effects, and other laboratories could not duplicate withdrawal. Therefore, the capacity of cannabinoids to produce abrupt withdrawal remains ambiguous. A second approach for assessing dependence is to precipitate an abstinence syndrome in chronically treated animals by administering an antagonist. The lack of a cannabinoid antagonist prompted earlier investigators to attempt precipitated withdrawal with opioid antagonists. Naloxone was reported to precipitate withdrawal in rats treated chronically with Δ^9-THC, although the symptomatology differed somewhat from that described for opioid dependence (Hirschhorn & Rosecrans, 1974; Kaymakcalan *et*

al., 1977). Fortunately, a selective and highly potent cannabinoid antagonist was developed recently (Rinaldi-Carmona *et al.*, 1994). This antagonist, SR 141716A, has proven to be effective in precipitating cannabinoid withdrawal. In one study rats were chronically infused with Δ^9-THC for 4 days and then administered the antagonist (Aceto *et al.*, 1995). A marked change in the Δ^9-THC-infused animals was evident approximately 10 minutes after the intraperitoneal injection of SR 141716A, and these effects subsided within 1 hour. The behavioral signs included head shakes, facial tremors, tongue rolling, biting, wet-dog shakes, eyelid ptosis, facial rubbing, paw treading, retropulsion, immobility, ear twitch, chewing, licking, stretching and arched back. The signs of facial rubbing and wet-dog shakes were quantified and found to be statistically greater than that observed in vehicle-infused rats. Similar results were observed by Tsou *et al.* (1995) who repeatedly injected rats with Δ^9-THC prior to an intraperitoneal challenge with SR 141716A. These studies provide convincing evidence that cannabinoids can produce physical dependence. The challenge is to understand the relationship between these animal models and the use pattern of cannabinoids in humans. A high priority for future research is to identify the neuronal systems which subserve the cannabis withdrawal syndrome. Manipulation of these systems may provide a means for treating individuals who seek assistance in terminating their cannabis use.

Pharmacokinetics and detection

Cannabis is usually smoked as a 0.5–1 g cigarette. The THC dose necessary to produce pharmacological effects in humans ranges from 2 mg to 22 mg for smoking (Martin, 1986). If only 10–25% of available THC enters the circulation when smoked, then the dose range is actually 0.2–4.4 mg. Animal studies have shown that the THC level in the brain is very small, with 1 % of the administered dose available at peak concentration (Agurell *et al.*, 1986). If humans have a similar distribution, then only 2–44 μg THC would penetrate the brain. Following inhalation, Δ^9-THC is rapidly absorbed into the bloodstream and redistributed. Initial metabolism takes place in the lungs and liver to 11-hydroxy-THC (11-OH-THC). This metabolite is somewhat more potent than Δ^9-THC and more readily crosses the blood–brain barrier. More extensive metabolism in the liver converts 11-OH-THC to many inactive metabolites, including 11-nor-carboxy-Δ^9-THC (THCCOOH), the most abundant metabolite in plasma and urine. A study by Huestis, Henningfield & Cone (1992) provides the first complete pharmacokinetic profile of THC and the appearance of metabolites during cannabis smoking. THC levels increase rapidly, peak prior to the end of smoking and quickly dissipate. Peak 11-OH-THC levels are lower than THC levels and occur immediately at the end of smoking. THCCOOH is detected minutes after smoking, and levels plateau for an extended period (Huestis *et al.*, 1992). Δ^9-THC can be detected in blood at 7 ng/ml and 18 ng/ml after a single inhalation of smoke from a 1.75 and a 3.55% THC marijuana cigarette, respectively (Huestis *et al.*, 1992). An entire cigarette will produce peak THC levels greater than 100 ng/ml (Lemberger *et al.*, 1972; Ohlsson *et al.*, 1980; Cocchetto *et al.*, 1981; Perez-Reyes, Owens & Di Guiseppi, 1981; Huestis *et al.*, 1992). Cannabis is also often consumed orally. Similar pharmacological effects to smoking result, but differences exist in the rate of onset and in the blood levels of cannabinoids. After oral ingestion, the levels of Δ^9-THC gradually increase over a period of 4–6 hours causing a delay in psychoactive effects (Wall *et al.*, 1983). 11-OH-THC is present in higher concentrations in blood after the oral route (Cone & Huestis, 1993).

Subsequent release of Δ^9-THC from lipid-rich tissues occurs slowly and produces a long elimination half-time. Estimates of elimination range from 18.7 hours to 4.1 days; the variability in half-life measures is due to the dependence of this measure upon assay sensitivity and timing of blood measurements (Cone & Huestis, 1993). Less variability is found in measurements of clearance. Recent data using sensitive detection techniques suggest that the elimination half-life in chronic users is actually 3–5 days (Johansson *et al.*, 1988). Conflicting reports exist for the clearance time of THC in light and chronic cannabis users. Lemberger & Rubin (1978) reported that the time to clear half of the dose from the body in a daily user (19–27 hours) is twice as fast than in an inexperienced user. Another study did not find significant differences in clearance rates between heavy and light users (Ohlsson *et al.*, 1982).

Since cannabinoids affect motor skills having a reliable measurement of impairment similar to the breath test for alcohol intoxication is desirable, but establishing a relationship between blood levels of THC or its metabolites and the degree of impairment has been difficult. This difficulty relates to the delay between peak blood concentrations and peak drug effects (Huestis *et al.*, 1992). Immediately after smoking, plasma levels are high while effects are low; whereas at later times, the situation reverses. Therefore, blood levels of THC could be useful for predicting impairment if the method and time of cannabis use is known. In the absence of this critical information, attempts to develop "cut-off" levels would have to be very conservative (i.e. the values would have to be rather high). Recently, models have been proposed to predict the time of cannabis exposure from plasma concentrations of THC and THCCOOH (Cone & Huestis, 1993). These models allow prediction of the elapsed time since cannabis use based on analysis from a single plasma sample. Additional research is needed to clarify the relationship between blood cannabinoid levels and behavioral effects.

Legal and moral concerns in the United States have led to increased efforts to detect cannabis use in the work place and in individuals whose performance is critical for general public safety. Initial screening tests are performed by immunoassay for the detection of cannabinoids in urine, and positive samples are verified by gas chromatography/mass spectrometry analysis. These assays were developed to detect the primary cannabinoid excreted in urine, which is THCCOOH. The development of "quick tests" for the detection of drugs of abuse results from the growing demand for simple, rapid and inexpensive on-site drug testing. The EZ-SCREEN® immunoassay cannabinoid test is highly sensitive for THCCOOH and has low cross-reactivity with other cannabinoids (Jenkins *et al.*, 1993). One of the most frequently asked questions is the length of time required for urinary levels to fall below detectable limits following smoking of a single cannabis "joint". Typically, THCCOOH can easily be detected 2–3 days following smoking of a single cannabis cigarette. Passive inhalation has become an attractive argument for explaining the presence of urinary cannabinoids. Yet Cone (1990) demonstrated that Herculean efforts were required in order for passive inhalation to produce detectable urinary levels of THCCOOH. Measurement of urinary levels of cannabinoids should be conducted solely for the purpose of determining whether an individual has used cannabis. Attempts at assessing impairment would require considerable knowledge of the circumstances surrounding the last use.

Effects on organ systems

Central nervous system

Since the brain is recognized as a principle target for cannabis, research has been conducted to study the effects of cannabinoids upon the central nervous system that extend beyond neurochemistry. The effects of cannabis on electroencephalographic (EEG) readings, cerebral blood flow (CBF) and brain morphology have been studied, as reviewed by Hall, Solowij & Lemon (1994) and Solowij (1996). Long term alterations in EEG recordings have been observed in cats, rats and monkeys exposed to cannabinoids (Hall *et al.* 1994). In one chronic study, monkeys were exposed to cannabis smoke for 6 months (Heath *et al.*, 1980). Serious subcortical EEG alterations were noted, with the amygdala, hippocampus and septal region most profoundly affected. Quantitative EEG studies of cannabis in humans have been performed since the 1970s, and most reported an increase in alpha power (usually relative power or alpha abundance), decreased alpha frequency and a decrease in beta activity following acute exposure to THC (Fink *et al.*, 1976). These results are consistent with a state of drowsiness. Struve & Staumanis (1990) provide a review of the acute and chronic effects of cannabis use on the EEG recording and evoked potential studies in humans. Recently, Struve, Staumanis & Patrick (1994) reported that THC produced significant elevations in absolute alpha power, relative alpha power and interhemispheric alpha coherence over frontal and frontal–central areas in chronic users. They referred to this phenomenon as alpha hyperfrontality. In users with very long exposure (>15 years), EEGs were characterized by increases in frontal–central theta activity in addition to hyperfrontality of alpha. These findings may suggest that there is a gradient of quantitative EEG change associated with long term cannabis exposure. Infrequent use did not produce persistent EEG change. With daily use, the topographic EEG becomes characterized

with hyperfrontality of alpha. At some unknown point after cumulative exposure there is a downward shift in maximal EEG spectral power from the mid alpha range to the upper theta/low alpha range. Exposure of 15–30 years results in increases of absolute power, relative power and coherence of theta activity over the frontal–central cortex. The relationship between EEG changes and performance on neuropsychological tests is not known.

Studies have also examined the effects of cannabis upon two measures of brain activity, cerebral blood flow and cerebral metabolic rate. Drug-induced changes in these parameters are thought to represent a change in brain function (Mathew & Wilson, 1993). One study showed that acute cannabis exposure in inexperienced users produced a global CBF decrease, whereas in experienced users CBF increased in both hemispheres, but primarily in the frontal and left temporal regions. The authors attributed the decrease in CBF in inexperienced subjects to their increased anxiety following cannabis administration, and the increase in CBF in experienced users was attributed to pharmacological effects of cannabis (Mathew & Wilson, 1992). The increased blood flow correlated with the levels of intoxication (Mathew *et al.*, 1992). Acute Δ^9-THC increases cerebral metabolic rate in humans and animals, although in humans the effects on the metabolic rate is probably limited to specific brain areas such as the cerebellum or prefrontal cortex (Margulies & Hammer, 1991; Volkow & Fowler, 1993). One study compared the acute effects of cannabis on three control subjects (who had used cannabis no more than once or twice per year) and three chronic subjects (who had used cannabis at least twice per week for at least 10 years) (Volkow & Fowler, 1993). Control subjects had an increase in metabolic activity in the cerebellum and pre-frontal cortex, and the subjects' subjective sense of intoxication correlated with the degree of increase in metabolism in the cerebellar cortex. Chronic users showed less changes in regional metabolism and reported fewer subjective effects, perhaps reflecting tolerance to the effects of cannabis.

Immune system

With efforts to use either cannabis or synthetic cannabinoids for therapeutic purposes, one should consider the potential effects on the immune system, especially in patients with a compromised immune system, as reviewed by Hall *et al.* (1994). Determining if cannabinoids impair the immune system is complicated by several factors. First, the majority of the studies have been conducted *in vitro* with animal and human cell cultures or *in vivo* in animals. Extrapolating these results to humans is further complicated by the very high doses of cannabinoids used in the studies. Secondly, the few *in vivo* human studies have produced conflicting results. Thirdly, very few epidemiological studies assessing disease susceptibility in heavy chronic cannabis users have been conducted.

The immune system is comprised of several components, including lymphoid tissues, such as the spleen and lymph nodes, the bone marrow and thymus where lymphocytes and other immune cells are made, and circulating lymphocytes. Immunity is either innate or acquired. Innate immunity involves immune responses that do not require previous sensitization and exposure to foreign substances. Actions of macrophages and natural killer cells are part of a host's innate immunity. Acquired immunity requires previous exposure to a foreign substance. These responses are mediated by two types of lymphocytes: B cells, which control humoral immunity, and T-cells, which control cell-mediated immunity. Humoral immunity occurs when B cells recognize antigens on the surface of foreign cells. The B cells proliferate and differentiate into cells which make and release antibodies and cells which circulate and respond to later exposure. The diverse responses of T cells produce cell-mediated immunity. T cells are antigen-specific. Some types of T cells directly kill virus-infected cells, while other types regulate the activity of B cells and macrophages.

Cannabinoids probably exert their actions through both cannabinoid receptor and non-receptor, or non-specific, mechanisms, since high concentrations are often needed to elicit an effect. A non-specific indication of an effect on the immune system is a decrease in weight of lymphoid organs (Munson & Fehr, 1983). Cannabinoids reduced the weight of the thymus in monkeys, and in high doses cannabinoids could affect the function of the stem cells and reduce the size of the spleen in rodents (Munson & Fehr, 1983).

The effects of cannabinoids on human, mon-

key and rodent macrophages have been studied both *in vivo* and *in vitro*. Cannabinoids can affect a macrophage's morphology, phagocytic and spreading ability, superoxide production and tumor necrosis factor and interleukin release. Rat alveolar macrophages were only moderately affected following 30 days exposure to cannabis smoke, with changes in morphology, superoxide production and oxygen consumption (Davies, Somberger & Huber, 1979). Human pulmonary alveolar macrophages obtained from cannabis smokers displayed a suppression of superoxide production (Sherman *et al.*, 1991). Macrophages from monkeys exposed to cannabis smoke for up to 1 year had altered morphology, including an increase in the number of vacuoles, and protein expression (Cabral *et al.*, 1991). THC adversely affected the phagocytic and spreading ability of macrophages from mouse peritoneal cultures (Lopez-Cepero *et al.*, 1986), and similar results occurred in human, mononuclear phagocyte cultures (Spector & Lancz, 1991). Cytokine, or interleukin, production in macrophages was also altered by THC. Interleukin 1 (IL1) bioactivity and release were increased (Klein & Friedman, 1990; Shivers *et al.*, 1994), and antiviral factor production was suppressed (Cabral & Vasquez, 1992). Since tumor necrosis factor (TNF) levels were either increased (Shivers *et al.*, 1994) or decreased (Zheng, Specter & Friedman, 1992; Fischer-Stenger, Pettit & Cabral, 1993) depending upon the type of cell culture, the effect of cannabinoids on cytokine levels is probably modulatory.

The effects of cannabinoids on the humoral immunity (production of B lymphocytes) and cell-mediated immunity (T lymphocyte production) are inconsistent. Conflicting *in vivo* studies were generated in the 1970s, with cannabinoids either suppressing human and monkey leukocyte numbers and functions (Gupta, Grieco & Cushman, 1974; Nahas *et al.*, 1974) or not affecting lymphocytes (Silverstein & Lessin, 1974; Lau *et al.*, 1976; Rachelefsky *et al.*, 1976). These studies were often performed with human patients without controlling life-style factors. In monkey studies conducted during the same period, blood cell mitogen responses and serum antibodies (IgG and IgM) levels were significantly reduced in monkeys chronically treated with THC for 6 months (Daul & Health, 1975). In another study, rhesus monkeys treated with THC for 3 weeks had elevated neutrophil levels; lymophocytes were not affected (Silverman *et al.*, 1982). A more recent study reported that in human outpatient cannabis abusers, the T cell CD4/CD8 ratio increased (Wallace *et al.*, 1988). CD4 and CD8 are cell–cell adhesion glycoproteins on the surface of T cells that act to stabilize the binding T cell receptors and antigen complexes on the target cell. However, Dax *et al.* (1989) demonstrated that in institutionalized patients receiving small amounts of cannabis for 3 weeks, white blood cell and subset lymphocyte counts and killer cell activity were unaffected. When the amount of THC and length of exposure time increased, IgG antibody levels decreased; IgD antibody levels increased, and IgA and IgM levels were unaffected (Nahas & Ossweman, 1991). From these studies, one can conclude that cannabis smoking appears to produce moderate disturbances in lymphocyte activity in humans and monkeys *in vivo*. However, the clinical relevance of these findings are uncertain (Hollister, 1988).

Cannabinoids also affect the function of cultured human lymphocytes. THC suppresses leukocyte migration (Schwartzfarb, Needle & Chavez-Chase, 1974) and lymphoproliferation (Nahas, Morishima & Desoize, 1977). Again, these effects occurred upon exposure to high doses. Spector & Lancz (1991) showed that 11-OH-THC suppressed natural killer (NK) cell activity. The mechanism for some of the effects of THC might involve adenylyl cyclase activity since THC suppresses agonist-induced cAMP in lymphocyte cultures (Diaz, Spector & Coffey, 1993). Cytokine levels in human lymphoid cultures either increased or decreased (Watzl, Scuderi & Watson, 1991).

Many reports provide evidence that cannabinoids affect the immune system of rodents. *In vitro* studies performed with rodent lymphocytes indicate that cannabinoids suppress antibody production (Klein & Friedman, 1990; Bacztnsky & Zimmerman, 1983), although the molecular mechanism for these effects remains unknown. B lymphocytes appear to be more sensitive to cannabinoid suppression than T lymphocytes (Klein *et al.*, 1985). Drug-induced suppression of antibody production is the most consistently reported observation in cannabinoid studies in the immune system. The effects of cannabinoids upon T lymphocyte proliferation do not always lead to suppression, suggesting

that cannabinoids act as modulators (Luo *et al.*, 1992; Pross *et al.*, 1992).

Several studies have suggested that cannabinoids decrease host resistance to infection. Cannabinoids caused enhanced mortality in rodents to *Lysteria monocytogenes* and Herpes simplex type II virus (Morahan *et al.*, 1979). Extrapolating these results to humans is difficult since drug doses that had the greatest effect were in the 100 mg/kg range. In more recent studies bacterial infections in mice have been examined using THC in the range of 5 mg/kg (Klein *et al.*, 1993, 1994). The effects of THC on resistance to infection depended on the dose and timing of injection. Animal studies confirmed that cannabinoids decreased antibacterial (Ashfaq, Watson & ElSohly, 1987) and antiviral activity (Cabral, Lockmuller & Mishkin, 1986) of the host immune system.

Cardiovascular

Cannabinoids also affect the cardiovascular system. THC can induce tachycardia, orthostatic hypotension and decreased platelet aggregation (Clark *et al.*, 1974; Schaefer *et al.*, 1979; Merritt *et al.*, 1980). In the rat, a transient pressor response is followed by hypotension and bradycardia (Dewey, 1986). Changes in the electrocardiogram include varied P and T waves and decreased ST segments (Johnson & Domino, 1971). Exposure to cannabinoids may aggravate pre-existing conditions such as angina and congestive heart failure. Hypotension and bradycardia result after prolonged exposure in humans (Benowitz & Jones, 1975). After high doses in humans, conjunctivae redden due to dilation of blood vessels and increased heart rate with a concomitant peripheral vasodilation (Dewey, 1986).

Recent work by Varga *et al.* (1995) implicates the involvement of the CB_1 receptor in the hypotensive action of anandamide. Anandamide produced a brief pressor response and a more prolonged depressor response. The depressor response only was inhibited upon administration of the cannabinoid antagonist SR 141716A. In addition, either cervical spinal cord transection or blockade of α-adrenergic receptors attenuated the depressor response. These results suggest that the pressor component of anandamide's cardiovascular response results from a peripheral action not mediated by the CB_1 receptor or the sympathetic nervous system. The depressor response is due to inhibition of sympathetic tone mediated by CB_1 receptors.

Human psychopharmacology

Cannabinoids produce a variety of acute psychological effects in humans, which are reviewed extensively by Hall *et al.* (1994). THC is rapidly absorbed after smoking, and acute peak effects appear between 30 and 60 minutes. When cannabis is ingested, the onset of action is slower, and subjective effects last for 5–12 hours without a clear peak. Acute subjective effects are dose-dependent. It is still unknown whether cannabis hinders performance and produces a hangover syndrome during the day after smoking. The subjective acute effects of cannabis are very diverse. One characteristic of cannabis use is a state of intoxication or euphoria and relaxation, followed by drowsiness, sedation and sometimes depression (Hollister, 1986). Other symptoms accompanying euphoria include alterations of motor control, sensory functions and cognitive (decision-making) processes (Nahas, 1993). Users of cannabis also claim that the drug heightens sensitivity to external stimuli, brightens colors and enhances music appreciation. At doses which produce a moderate level of intoxication, a wide range of learned and unlearned behaviors, including simple motor tasks and complex psychomotor and cognitive tasks were affected (Chait & Pierri, 1992). Cannabis adversely affects gross and simple motor tasks (body sway and hand tremor), psychomotor behavior (rotary pursuit, Digit Symbol Substitution, reaction time, accuracy in divided attention and sustained attention) (Chait & Pierri, 1992). Cannabis had weak effects on simple reaction time and inconsistent effects on hand–eye-coordination. Data from Heishman *et al.* (1990) indicate that cannabis can impair complex human performance in arithmetic and recall tests up to 24 hours after smoking.

Scientific evidence suggest that marihuana impairs memory and learning. Δ^9-THC causes its greatest and most consistent effects in short-term memory, as measured in free recall of previously learned items. The major impairment by cannabis in free recall studies produces substantial increases in memory intrusions (Chait & Pierri, 1992). Neither immediate and sustained attention nor controlled retrieval from semantic mem-

ory were affected. Thus, THC probably impairs acquisition and working memory but not retrieval processes. The effects of cannabis upon recall in the digit span, recognition and paired-associate memory performance tasks have been inconsistent (Chait & Pierri, 1992; Schwartz, 1993). Generally, cannabis did not affect the retrieval of previously learned facts. Although the acute effects of THC on memory appear modest, one should consider the effects of chronic use upon adolescent development.

THC does alter time perception, producing an overestimation of elapsed time (Chait & Pierri, 1992). Associated with the altered time sense is temporal disintegration, which is defined as difficulty in retaining and coordinating memories and perceptions relevant to a goal the user is perusing (Melges *et al.*, 1970). The effect of changed time perception and short-term memory disruption might be reflected in decreased driving and occupational skills, but evaluation of work productivity in chronic users has not detected major decrements in work performance (Hollister, 1986).

Impairment of both cognition and motor control has been documented in a laboratory setting and proposed as a contributor to accident and traffic fatalities (Aussedat & Niziolek-Reinhardt, 1993) and non-vehicular accidents (Soderstrom *et al.*, 1993). However, based upon a review of the literature, no clear relationship has been shown between cannabis smoking and either seriously impaired driving performance or the risk of accident involvement. The extent that cannabis contributes to traffic accidents is not known with certainty. Results from laboratory studies and driving simulations are reviewed extensively by Chesher (1995) and Robbe (1994). Laboratory studies have shown performance impairment occurring after inhaled doses of cannabis as low as 40 μg/kg. Cannabis produces a dose-dependent impairment on specific skills, which become pronounced after 100–200 μg/kg doses. In particular, tracking, divided attention and vigilance tests performance are affected by THC. In contrast, results from driving simulator and closed-course tests surprisingly indicate that THC in single-inhaled doses up to 250 μg/kg has relatively small effects on driving performance. Explaining the disparity in results obtained in laboratory studies and in driving simulations is difficult. Recently, Robbe (1994) performed a series of studies which evaluated the effects of cannabis smoking on actual driving performance and compared these results to the effects of alcohol on driving. Several driving tests were employed including maintenance of a constant speed and lateral position during uninterrupted highway travel, following a lead car with varying speed on a highway and driving in a city. Cannabis produced a moderate degree of impairment, which was related to the THC dose. At a dose of 300 μg/kg THC impaired road tracking ability and slightly impaired the ability to maintain a constant headway when following another car. A low THC dose (100 μg/kg) did not impair driving ability in the city to the same extent as a blood alcohol concentration of 0.04%. Drivers under the influence of marijuana tended to overestimate the level of impairment and compensate by concentrating on driving and/or slowing down. In contrast, drivers under the influence of alcohol tended to underestimate the effects of alcohol and not make allowances for impairment. Several studies have also attempted to determine the incidence of cannabis involved in road crashes in which the driver had consumed cannabis and was responsible for the collision. Three studies have reported that cannabis-bearing drivers were no more responsible than the non-drug-bearing drivers (Williams *et al.*, 1985; Terhune *et al.*, 1992; Drummer, 1994). This finding must await clarification until sample sizes are greatly increased. Robbe (1994) concluded that while campaigns to discourage the use of cannabis by drivers are warranted, concentrating upon cannabis alone may not be in proportion to the safety problem it causes.

Several factors complicate the interpretation of cannabis-induced impairment, such as co-use with other drugs, variability among individuals, development of tolerance and intrinsic difficulties in conducting a systemic evaluation in the general population. Cannabis is often co-abused with other drugs, such as alcohol. Co-use of cannabis with alcohol (Wechsler *et al.*, 1984) or phencyclidine (PCP) (Poklis, Maginn & Barr, 1987) might augment cannabis' effects. Results indicate that performance disruption was greater for alcohol-induced impairment in combination with cannabis (Hollister, 1986). It has been reported that ethanol-induced dose-dependent decrements in performance skill required for automobile driving were further exacerbated by cannabis (Perez-Reyes *et al.*, 1988). Tolerance does develop during chronic exposure to high

quantities of cannabis, but the degree of tolerance following intermittent exposure to cannabis is less definitive (Hollister, 1986). Detecting cannabis intoxication by motor performance in an experienced user may be difficult unless a complex performance task is assessed or if the user has had experience in the task (Chait & Pierri, 1992). Cannabis intoxication in an inexperienced user is readily detectable by many performance tests. Establishing a degree of correlation between the level of impairment and blood concentrations of cannabinoids would aid in determining causality in accidents. However, given the confounding factors discussed above, it is unlikely that measures of Δ^9-THC and its metabolites will become standards for intoxication.

Human studies have been conducted to determine if a state dependent learning (SDL) effect exists for cannabis. The first evidence of a cannabis-induced state dependency was reported by Abel (1970). Subjects learned narrative material while exposed to cannabis and were tested in a sober or cannabis-intoxicated state. A greater deficit of recall was recorded for subjects tested in the sober state. Evidence also exists that the SDL effects of cannabis are most apparent in tasks using sequential memory (Hill *et al.*, 1973; Stillman *et al.*, 1974). The SDL effect for cannabis is observed in memory tasks rather than psychomotor or adversely motivated tasks (Järbe *et al.*, 1993). Difficult tasks, such as active recall, are also affected by SDL (Järbe *et al.*, 1993). In order to determine the influence of the frequency of use upon cannabis' effects on memory, one study differentiated between heavy and social users (Cohen & Rickles, 1974). Subjects in the heavy-user group average smoking cannabis five to six times per week for a year. The social-user group smoked at weekends. The frequency of use did have profound effects on the SDL effects of cannabis. In recall tests, social users did exhibit state-dependent effects, whereas heavy users did not. The heavy-user group performed equally well whether intoxicated or not, and they performed better in recall than social-users.

Since one of the well known acute effects of cannabis is to impair cognitive functioning, it has long been suggested that chronic cannabis use may cause lasting cognitive impairments. Assessing the chronic effects of cannabis or any other psychoactive drug on cognitive functioning is often difficult since many factors other than drug use must be controlled. Difficulties encountered when attributing cognitive effects to psychoactive drugs include determining levels of cognitive impairment which might have preceded drug use, determining the duration and frequency of drug use and taking into account effects of multiple drug use. It has been proposed that chronic use might result in long-term memory impairment (Schwartz, 1993). However, previous reviews have generally concluded that evidence is insufficient to conclude that long-term use of cannabis produces lasting gross cognitive impairment (Wert & Raulin, 1986). Pope *et al.* (1995) and Solowij (1996b) have reviewed recent, more methodologically rigorous research which used improved test procedures and electrophysiological methods. These findings provide evidence that cannabis produces complex and subtle impairments, which are related to the duration of cannabis use. Impairments appear specific to higher cognitive functions, such as the organization and integration of complex information involving attention and memory processes (Solowij, 1996b). It has been hypothesized that long-term cannabis use impairs the frontal lobe, an area of the brain which functions in the temporal organization of behavior. This hypothesis is consistent with the altered perception of time and with cerebral blood flow studies which demonstrate greatest effects in the frontal lobe region. Recent studies also suggest that impairment assessed by sensitive measures of brain function can be detected after only 5 years of use. Not all individuals are affected equally by long-term use. Often the effects are subtle, but one should not underestimate the effects of even subtle impairment of cognitive functioning on daily life.

Great interest has been generated in the effects of cannabis upon adolescent development and educational performance and production of an "amotivational syndrome". A modest statistical relationship may exist between cannabis and other illicit drug use and poor educational performance (Schwartz, 1993). Some individuals suffer no memory impairment at all, whereas those individuals who already have a learning disability are more susceptible to memory disruptions than a gifted student group (Schwartz, 1993). Attempts to verify the existence of an "amotivational syndrome" have failed (Dewey, 1986; Hollister, 1986; Foltin *et al.*, 1989; Foltin *et al.*, 1990). The lack of motivation observed in

some individuals more probably results from psychosocial problems and polydrug use rather than solely cannabis use (Taschner, 1983). Additional research should address the impact of long-term cannabis use cannabis on cognitive development in adolescents.

Since THC produces diverse psychological effects in humans, it has been suggested that cannabinoids might induce psychopathological states (Talbott & Teague, 1969; George, 1970). However, identification of a specific "cannabis psychosis" even in chronic, heavy users has not occurred (Dewey, 1986; Hollister, 1986; Thornicroft, 1990). Cannabis does appear to worsen symptoms of some pre-existing mental disorders, such as schizophrenia (Negrete, 1993). Even though paranoid schizophrenics recognize the worsening of their disorder with cannabis use, many still continue to try to self-medicate themselves with the drug. Cannabis increases hallucinations and delusions and produces inconsistent results on the symptoms of social withdrawal and lethargy. While some investigators believe that cannabis use does lead to the development of schizophrenia, conclusive evidence does not exist that cannabis is a causative factor in the development of schizophrenia (Allebeck, 1993; Negrete, 1993). Individuals abusing cannabis who also develop psychiatric problems may suffer from rapid onset schizophrenia (Allebeck, 1993). Since most of these individuals are polydrug users, it seems more likely that cannabis or any of the other abused drugs might act as a trigger for precipitating latent schizophrenia. The relative risk of developing psychiatric problems in the general population of cannabis users is apparently very small. Proper studies comparing the development of disorders in abusers and non-abusers have not been performed. Yet given the world-wide and prevalent use of cannabis, one would expect to see more reported cases of cannabis-induced psychiatric disorders if cannabis readily caused them.

Human tolerance and dependence

In the late 1960s and early 1970s, there was considerable confusion regarding the development of tolerance to smoking cannabis. The well known phenomenon that many newcomers required several smoking episodes before experiencing the cannabis "high" led to the hypothesis that "reverse tolerance" developed. The notion that tolerance could then develop to cannabis' psychotomimetic effects formed the basis of the proposed "reverse-reverse tolerance". There is no doubt that many factors, other than the inherent properties of Δ^9-THC, are contributors including potency of the cannabis, expectations, environmental influences, individual differences and frequency of use, to name just a few. However, convincing evidence exists for the development of tolerance to Δ^9-THC in humans (Jones, Benowitz & Bachman, 1976), as was described above for animals. Tolerance developed to a variety of Δ^9-THC's effects following oral administration including cannabinoid-induced decreases in cardiovascular and autonomic functions, increases in intraoccular pressure, sleep disturbances and mood changes (Jones *et al.*, 1976). Results are less conclusive for behavioral tolerance. To achieve behavioral tolerance, high doses of Δ^9-THC were administered for a sustained period of time. In one study, tolerance to the subjective effects of Δ^9-THC developed after oral administration (10 mg) for several days; greater tolerance developed with increased amounts of the drug (Jones, 1983). Thus, if the doses of Δ^9-THC are small and infrequent, little behavioral tolerance develops. High doses must be given for long periods of time to produce tolerance.

Although it is established that chronic cannabis use does not result in severe withdrawal symptoms, numerous case reports attest to development of dependence (Jones, 1983). Several early reports came from countries where potent cannabis was used for long periods of time. Upon deprivation of cannabis, users experienced auditory and visual hallucinations and irritability (Fraser, 1949). Since that report, the development of tolerance and dependence have been studied under rigorous and controlled conditions (Jones & Benowitz, 1976; Jones *et al.*, 1976; Jones, Benowitz & Herning, 1981; Jones, 1983). In one study, a 30 mg dose of cannabis extract or Δ^9-THC was administered orally approximately 6 times per day for up to 21 days. The most prominent symptoms upon cessation of administration were increased irritability and restlessness. Other symptoms, although variable, included insomnia, anorexia, increased sweating and mild nausea. Objective symptoms were increased body temperature, weight loss and hand tremor. Re-administration of a cannabis cigarette or oral Δ^9-THC alleviated the objective and sub-

jective effects, suggesting the establishment of a withdrawal symptom.

Potential therapeutic uses

The prevalence of cannabis use has resulted in intense efforts of cannabis research for the past several decades. Attempts have been made to discern the pharmacology of cannabis and the mechanism of action producing the psychoactive effects. In addition to exploring the euphoric effects of cannabis, emphasis has been placed upon the drug's therapeutic potential. Early crude preparations of cannabis treated allergies and migraines and facilitated childbirth (Mechoulam, 1986). The effective component, Δ^9-THC, was also used for alleviating pain, glaucoma, muscle spasticity, bronchial asthma and nausea (Hollister, 1986). However, the lack of evidence that cannabinoids are better than other drugs currently in use limits their clinical usefulness. In addition, separating the undesired side effects of cannabis from the therapeutic effects has proved difficult. Schedule II drugs require extensive record-keeping and cause other administrative problems. Pharmaceutical companies have marketed only Δ^9-THC, which is used primarily as an antiemetic for cancer chemotherapy patients. The development of a cannabinoid analog possessing greater pharmacological selectivity is an important aim for future cannabinoid research.

Although many useful probes for determining the underlying mechanism of action for cannabinoids have been produced, no clinically relevant compound has emerged from the progress. The inability to separate the various pharmacological and psychoactive properties of the compounds remains the greatest impediment. Cannabinoids have generated interest over the centuries for their alleged ability to treat a wide range of disorders. Possible therapeutic uses include treatment of bronchial asthma, nausea, vomiting, pain, convulsions, glaucoma, muscle spasticity and loss of appetite (Hollister, 1986). Cannabinoids also represent a novel way to treat disorders not responding to traditional agents or therapies. Current debate in the United States centers upon the possible legalization of cannabis for medicinal purposes. Proponents of legalization believe that the availability of THC would eliminate the need for the crude plant product. While there may be some merit in legalization arguments, the development of a potent and selective cannabinoid possessing greater efficacy than current drugs would, of course, end the ongoing debate.

Cannabis has been used most frequently for treating refractory nausea and vomiting. In 1987, the United States Food and Drug Administration approved dronabinol, a Δ^9-THC formulation in sesame oil, for treatment of chemotherapy-induced nausea and vomiting not responding to other agents. Dronabinol has been useful, although some patients dislike the psychotropic effects and somnolence. Δ^9-THC has gained orphan status by the FDA to treat nausea from chemotherapy and to stimulate appetite in AIDS patients. Results from clinical trials have suggested that the drug improves appetite (Plasse *et al.*, 1991). Nevertheless, one should remember that extensive animal studies indicate that cannabinoids adversely affect the immune system. Should a drug with possible immunosuppressive properties be given to patients who already have a compromised immune system? Only future research and more extensive clinical evaluation will determine if Δ^9-THC truly benefits these individuals.

Drug development has also focused upon the potent antinociceptive properties of cannabinoids. Great progress would be made in synthesizing an analgesic agent lacking the side effects and abuse liability of opioids. Unfortunately, cannabinoids produce antinociception at doses that also elicit other behavioral effects, such as sedation, hypothermia and catalepsy. Cannabinoids have a distinct pharmacological profile from the opioids and may act through a different mechanism for alleviating pain. Recent research demonstrated that a kappa receptor antagonist, nor-binaltorphimine (nor-BNI), blocked cannabinoid-induced antinociception, but did not affect the other behaviors (Smith, Welch & Martin, 1993). Perhaps this compound could be used to disseminate the mechanism of action for cannabinoid analgesia.

Summary

Great progress has been made during the past 10 years regarding our understanding of the mechanism of action of cannabinoids. A specific cannabinoid receptor has been identified and cloned, and its distribution has been mapped throughout the central nervous system. The

cellular mechanism of action of cannabinoids has been more clearly defined. The discovery of anandamide as an endogenous ligand for the cannabinoid receptor creates the possibility of discovering a novel neurochemical system. The actions of cannabinoids and anandamide can better be elucidated with the recent discovery of an antagonist for the receptor. These advancements provide powerful tools for future research and should contribute to the expansion of our knowledge of the cannabinoid field.

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