

REVIEW ARTICLE

NEUROBIOLOGY OF CANNABIS ADDICTION

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( Received on May 20, 2008 )

**Abstract :** Cannabis has emerged as a common substance of abuse and dependence and the peculiarities associated with this widely available and used substance has triggered substantial research in this field. The earlier held concept of rather benign nature of this compound as a substance of abuse and dependence has changed as a result of the ongoing clinical and research findings. Cannabis has been found to have multiple physical and mental effects in human beings. But still a lot remains to be answered regarding the basis for the development of dependence on cannabis. However, the discovery of various cannabis receptors and their endogenous and synthetic ligands have added fuel to the ever growing interest in this substance. Various hypotheses have been postulated in this regard based on the findings of both the animal and human studies which serve as potential explanations to the observations. These findings have helped in the better understanding of the issue and have provided substrate for the clinical application.

**Key words :** anandamide      cannabis      cannabinoids      receptors

INTRODUCTION

Drug abuse is a major public health problem. Regular consumption of addictive substances has numerous health, family and socioeconomic facets. Of the many such substances, cannabis is far the most commonly used illicit drug world wide. Cannabis is derived from Indian hemp plant '*Cannabis Sativa*'. This plant contains over 400 chemical substances, with about 60 responsible for its unique effect. The

main psychoactive ingredient is  $\Delta^9$ -tetrahydrocannabinol (THC). The strength of cannabis depends on how much THC it contains. The concentration of the THC varies considerably in different plants and preparations and also between the parts of the plant that are used. The THC content is highest in the flowering tops, declining in the leaves, lower leaves, stems, and seeds of the plant. Cannabis is used in various forms such as bhang, ganja, charas, hashish, hashish oil and sinsemilla. Concentration of

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THC varies with different preparations like bhang (1%), ganja (1–2%), sinsemilla (up to 6%), hashish (8–14%). Hashish oil is the most concentrated form of cannabis. Its concentration can range from about 15–50%. All forms of cannabis are mind-altering. Investigators have always been at a serious disadvantage in trying to address the science of plant material abuse. It is impossible to examine the pharmacological and toxicological consequences of human and animal exposure to hundreds of compounds simultaneously (1). Thus, much attention has been devoted to  $\Delta^9$ -THC. The present aim is to focus mainly on the more recent literature in the field of neurobiology of cannabis addiction.

#### Pharmacokinetics of cannabinoids

The pharmacokinetics of cannabinoids are reviewed by several investigators (1–5). Cannabis may be smoked in a “joint”, which is the size of a cigarette, or in a water pipe. The bioavailability of the dose and the resulting kinetic profiles for plasma are greatly affected by the route of administration. Depending how cannabis is used, the body absorbs, metabolises and get rid of THC differently. The pharmacokinetics of THC vary as a function of its route of administration (3). When it is smoked, THC is rapidly absorbed into the lungs and enters the blood stream within minutes. Effects are perceptible within seconds and fully apparent in a few minutes and decline very rapidly to about 5–10% of their initial level within one hour. The potency of THC and time used for smoking are important determinants of bioavailability and plasma concentrations. Furthermore, the puff duration, volume of smoke inhaled, and holding of the breath after inhalation are also important factors contributing to the amount of cannabinoids

absorbed. Bioavailability after oral ingestion is much less and ranges from 4–12% as THC takes much longer to be absorbed into the blood. Blood concentration reaches 25–30% of those obtained by smoking the same dose, partly because of first-pass metabolism in the liver, so the onset of effect is delayed (0.5–2 hours). Once absorbed, THC and other cannabinoids are rapidly distributed to all other tissues at rates dependent on the blood flow. Cannabinoids are highly lipid soluble and accumulate in fatty tissues, reaching peak concentrations in 4–5 days. They are released back into other body compartments, including the brain. Because of the sequestration in fat, the tissue elimination half-life of THC is about 7 days, and complete elimination of a single dose may take up to 30 days (2). With repeated usage, high levels of cannabinoids can accumulate in the body and continue to reach the brain. Within the brain, THC and other cannabinoids are differentially distributed. Mainly, high concentrations are reached in neo-corticals, limbic, sensory and motor areas.

Cannabinoids are metabolised in the liver. THC is rapidly transformed, first by the hepatic cytochrome P450 enzyme system to 11-hydroxy- $\Delta^9$ -THC (11-hydroxy-THC), which is then oxidised by alcohol dehydrogenase to 11-nor- $\Delta^9$ -THC-9-carboxylic acid (Carboxy-THC). 11-Hydroxy-THC is a psychoactive metabolite, whereas carboxy-THC is devoid of psychoactivity. Two-thirds of the metabolites of THC are excreted into feces, the rest via the kidneys. There is also a significant enterohepatic circulation of THC. The primary urinary metabolite is Carboxy-THC, which is present both as the free acid and its glucuronide conjugate. Other metabolites formed in urine are 8-beta-hydroxy- $\Delta^9$ -THC and a large number of acid metabolites, most of which are

unidentified (6). Elimination half lives varies from naive individuals in whom it is about 50–57 hours to chronic users in whom it is about 19–27 hours (7).

#### **Cannabis and brain: How does it act?**

The effects of psychoactive constituent component  $\Delta^9$ -THC have been under scrutiny for years. Cannabis and THC exert manifold actions on a number of organ systems including the gastrointestinal system, the cardiovascular system, the respiratory system, the autonomic system and the central nervous system. Ingestion of cannabis produces a sense of enhanced well being, and relaxation with an intensification of ordinary sensory perceptions above the psychotropic threshold. Along with the pleasurable effects cannabis also produces certain unwanted psychic effects most important of which are anxiety and panic attacks (8–11). Cannabinoids, the compounds structurally similar to  $\Delta^9$ -THC, have been a great help in understanding the mechanism of action of cannabis. How these compound act remained an unsolved mystery for the science for many years. However, the molecular mechanisms underlying these events have only recently been identified. Over the last few decades tremendous progress has been made in the understanding of the cannabinoid receptors, both centrally and peripherally and is designated as receptor CB1 (12) and receptor CB2 (13, 14) and also two putative endogenous ligands, anandamide (15) and 2-arachidonyl glycerol (16), and the development of a CB1 receptor antagonist (17). Cannabinoids act via two type of receptors i.e. CB1 and CB2 types in the brain. The CB1 receptor mediates most of the central action of the Cannabinoids. CB1 is larger than CB2 with an additional 72 amino acid residues. The CB1 receptor is

a heptahelical G-protein -coupled receptor (GPCR) that is coupled primarily through pertussis toxin-sensitive  $G_i/G_o$ -type G proteins to signal transduction process including inhibition of adenylyl cyclase, activation of  $K^+$  channels, inhibition of  $Ca^{2+}$  channels, and stimulation of mitogenic kinases (18, 19). The CB1 receptors are most abundant GPCR in the brain, with levels approximately 10 times higher than most other GPCRs (20–22). Anatomical studies have revealed dense localization of CB1 receptors in the basal ganglia, hippocampus, cerebral cortex, and cerebellum, with lower levels present in many other brain regions including thalamus, hypothalamus, amygdala, and periaqueductal grey (21, 23–25). This distribution is consistent with the behavioral effects of cannabinoids, which include memory impairment, antinociception, catalepsy, hypomotility, and hypothermia, as well as alterations of mood and perception in humans (26). The lipid derivatives anandamide and 2-arachidonylglycerol act as endogenous ligands for CB1 receptors. They act as retrograde synaptic mediators of the phenomenon of depolarization induced suppression of inhibition or excitation in hippocampus and cerebellum. Cannabinoid receptors, i.e., CB1 and CB2, enzyme anandamide amidohydrolase (AAH) concerned with the hydrolytic breakdown of anandamide, and the carrier protein anandamide transporter (ANT) involved in the transportation of anandamide across the cell membrane are the biological targets for the endocannabinoids. The last two of these are responsible for the termination of the action of the anandamide. Anandamide along with most of the analogs have got a significant selectivity with high affinity for the CB1 receptors and modest to low affinity for the CB2 receptors (27).

Anandamides also cause inhibition of human brain muscarinic acetylcholine receptor (mAChR), which is involved in the memory function and is inhibited by arachidonic acid as well. The classes of neurons that express high levels of CB1 receptors are the GABAergic interneurons in the hippocampus, amygdala and cerebral cortex, which also contain neuropeptide cholecystinin.

Activation of CB1 receptors leads to inhibition of the release of amino acid and monoamine neurotransmitters. Central effect of cannabinoids includes disruption of psychomotor behaviour, short term memory impairment, intoxication, stimulation of appetite, antinociceptive actions (14). Cannabinoids have also been found to be important in the brain's regulation of the sleeping process. This finding comes from the observations in experimental animals, especially rats in studies using SR 141716, a cannabinoid receptor antagonist. SR 141716 is like "anti-marijuana" in that it enhances the same memory functions that natural brain cannabinoid anandamide and THC inhibit through the action on cannabinoid receptors. This compound improves short-term memory in rodents and also leads to interruption of the sleep cycle, causing a deficit in both the short wave and REM sleep. Anandamide has also been shown to lower the blood pressure and the heart rate through its action on the CB1 receptor, an effect that is blocked by the CB1 antagonist SR 141716. The possible explanation of this effect is vasodilatation produced by anandamide through the sympathetic system (17, 28).

Latest research in the field has revealed that  $\Delta^9$ -THC is cytotoxic causing shrinkage of neurons and DNA fragmentation in hippocampus. CB2 receptors which

have got a high preponderance in the haematopoietic tissue including the spleen serve as the molecular basis for the immunosuppressive action of cannabis. As mentioned above, the CB1 receptors act through the agency of a number of second messenger molecules. They cause an inhibition of adenylate cyclase, inhibition of N- and P/Q- type calcium channels. They have also been implicated in the activation of potassium channels and activation of the mitogen-activated protein kinase. The CB2 receptor act through the agency of adenylate cyclase and mitogen activated protein kinase, causing inhibition of the earlier and activation of the later (29). Around two centuries ago cannabis was being used for the management of the migrainous headache. The possible mechanism of action postulated for this effect of cannabis is inherent in the ability of cannabis in release of the neurotransmitter serotonin, which is the cause of the headache experienced in this condition. Although the research in this field has failed to prove the kind of role cannabis plays in this condition-that of an analgesic or anti emetic (30).

Among various effects produced by cannabinoids in experimental animals, one is alteration in the body temperature which is associated with the changes in the activity of brain 5-hydroxytryptamine (5-HT) neuronal system. Fluoxetine, a drug with an action involving 5-HT when administered prior to THC, abolishes its action on the body temperature. In case it is administered subsequent to THC administration, it potentiates hypothermia induced by THC. This action of cannabinoids includes interactions with the dopaminergic system as well. Possible role of cannabis in production of psychosis can through more light on the likely mechanism of action of the substance. Although production of

psychotic symptoms during acute and chronic use may be a mere precipitation of these features in predisposed individuals rather than being direct result of cannabis action, the cognitive distortion involved in these subjects is thought to be due to the deficits in sensori motor gating or filtering of sensory information. Moreover, advances have been made in defining the role of second messenger systems at the cellular level. Along with the adenylyl cyclase/cAMP second messenger system other second messengers have also been implicated in the action of cannabinoids. These include IP3/DAG and cGMP pathways. However, these actions are limited mainly in extra cranial sites and cAMP/adenylyl cyclase system continues to be the major secondary messenger system in the brain and spinal cord (31). Cannabis induced modulation of neurotransmitter is also mediated through the agent of various ion channels following the sequence of depolarization or hyperpolarisation.

It has been postulated that there is a opposing activity of mu- and kappa-opioid receptors in modulating reward pathways that forms the basis for the dual euphoric-dysphoric activity of THC. Preclinical studies have shown that deletion of kappa receptors ablates THC place aversion and furthermore unmasks THC place preference and absence of microreceptors abolishes THC place preference in rodents (32).

#### **Psychoactive and Pharmacological effects in humans**

Although the use of cannabis use occurs worldwide, its psychological and health effects are not well understood and remains under debate, with opinions on its risks polarised along the times of proponents' view on what its legal status should be or otherwise. The pharmacological and

toxicological consequences of cannabis exposure are likely due to numerous compounds cannabinoids and non-cannabinoids in nature. Breakthroughs in the past few years have greatly increased the understanding of cannabinoids ubiquitous effects on biological system (33). Cannabinoids produce a variety of acute psychological effects in human (1, 3, 34–36). One characteristic of cannabis use is a state of intoxication or euphoria and relation followed by drowsiness, sedation and some times depression. Other symptoms accompanying euphoria include alterations of motor control, sensory functions and cognitive (decision-making) processes. Along with the pleasurable effects cannabis also produces certain unwanted psychic effects most important of which are anxiety and panic attacks (6, 9). Delirium associated with the memory impairment disorientation has been observed in a few cases of cannabis overdose. However there is no biochemical model that could explain this particular finding. Dependence on cannabis is a potential development especially in chronic, heavy users or those predisposed to the addictive behaviour. Cannabis dependence follows different set of manifestations as it rarely leads on to the overtly harmful consequences to the individual contrary to what is seen with other drugs of abuse. Cannabis has been found to promote non-depressive thoughts and feelings in the users and its anti-depressant effect has been confirmed in many studies, which may serve as a possible factor for the regular use of the substance. Cannabis has been implicated as one of the gateway drugs to the potentially more harmful drugs, which serves as the basis for anti-cannabis slogans. Paranoia attacks as well as stress relief are other reported effects of cannabis; however, there is still no consensus on these issues.

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 (37). Pope et al. (1996) reported a drug residue effect on attention, psychomotor tasks and short term memory during the 12–24 hour after cannabis use. Electro-physiological and neuropsychological studies show that it may produce more reversible impairment of memory, attention and the organisation and integration of complex information which under effect (38). Cannabinoids have been shown to impact different stages of memory including encoding, consolidation, and retrieval. Several mechanisms, including effects on long-term potentiation and long-term depression and the inhibition of neurotransmitter release, have been postulated as the underlying causes for these effects of cannabinoids (39).

Cannabis act on specific cannabinoid receptor in the brain. Cannabis, moreover, has a long half life gets accumulated in body tissues and is being detectable in urine upto 6 weeks of last consumption. Thus subtle effects on nervous system functions may persist after the immediate pleasurable and other obvious psychological effects have disappeared. There is still a debate whether acute or chronic, cannabis use may lead to permanent impairment of cognitive function and behaviour because conclusive evidence is either way lacking (38, 40).

Great interest has been generated in the effects of cannabis upon adolescent development and educational performance and production of an 'amotivational syndrome'. Attempts to verify the existence of an "a motivational syndrome" have failed (7, 26, 30, 31). The lack of motivation

observed in some individuals more probably results from psychosocial problems and polydrug use rather than solely cannabis use (32). Additional research should address the impact of long-term cannabis use on cognitive development in the adolescents.

Since THC produces diverse psychological effects in humans, it has been suggested that cannabinoids might include psychopathological states (32, 33). However, identification of a specific cannabis psychosis even in chronic heavy users has not occurred (7, 26, 32). Cannabis does appear to worsen symptoms of some pre-existing mental disorders, such as Schizophrenia. Conclusive evidence does not exist that cannabis is a causative factor in the development of Schizophrenia (6, 8). Since most of these individuals are polydrug users, it seems more likely that cannabis or any other abused drugs might act as a trigger for precipitating latent Schizophrenia. Cannabis abuse has also been associated with anxiety and depressive disorders. The relative risk of developing psychiatric problems in the general population of cannabis users is apparently very small.

#### **Biological theories of cannabis addiction**

Cannabis has been associated with a low addiction potential as compared to the other drugs of abuse like opioids and alcohol. But it would be inappropriate to underestimate its abuse and dependence liability as cannabis addiction has been observed in about 9% of the users, some studies documenting even a figure in excess of 50% of users who have "impaired control" over their use of cannabis. Tolerance to cannabis develops rapidly after a few doses and reverses rather rapidly after abstinence. The acute effects of cannabinoids as well as the development of tolerance are mediated by G protein coupled cannabinoid receptor. Studies regarding the development of abstinence symptoms following use

of cannabis in form of smoking have demonstrated presence of withdrawal symptoms which include restlessness, irritability, mild agitation, insomnia, nausea, sleep disturbance, sweats and intense dreams. These are mild and last for a short period only (34, 35).

Common features of withdrawal syndromes produced by several drugs of abuse including elevations in extracellular corticotropin-releasing factor (CRF) levels in the mesolimbic system and a marked inhibition of mesolimbic dopamine activity have been reported during cannabinoid withdrawal. This alteration of limbic system CRF function may mediate the stress-like symptoms and negative affect that accompany cannabinoid withdrawal. In agreement with this hypothesis, the spontaneous firing rate of ventral tegmental area (VTA) dopamine neurons is reduced during cannabinoid abstinence which is likely related to the aversive and dysphoric consequences of cannabinoid withdrawal. Cannabinoid withdrawal is associated with compensatory changes in the cAMP pathway. Initially, acute activation of CB1Rs inhibits adenylyl cyclase activity. The cerebellum plays a crucial role in the somatic expression of THC withdrawal. Cannabinoid abstinence is markedly reduced when cAMP-dependent protein kinase is activated in the cerebellum (36).

Recent studies suggest that anandamide is released from the neurons following depolarization through a mechanism requiring calcium-dependent cleavage from a phospholipids precursor in neuronal membranes. This is followed by rapid uptake into the plasma and hydrolysis by fatty acid amidohydrolase. Dopamine is the neurotransmitter in the brain that is associated with the rewarding centers. These

centers have a close association with the limbic system, the region associated with the control of emotions and behaviour. Ability of a drug to influence these dopaminergic pathways is a strong determinant of the ability of the drug to possess abuse potential, through its reinforcing actions. Cannabinoids lead to an increase in the dopaminergic activity in the ventral tegmental area-mesolimbic pathway. This action of cannabinoids has been implicated as the basis for reinforcing and abuse properties of cannabis. This final common pathway for reinforcing action is shared by a number of other drugs of abuse including morphine, ethanol and nicotine<sup>9</sup>. Moreover, CB1 receptors have been assigned a functional role in the cannabis addiction because of their location in the regions of the brain associated with the reward generating areas, namely the mesolimbic-mesocortical dopaminergic system. This has been supported by the immunohistochemical investigations (37).

Furthermore, studies carried out with the cannabinoid receptor antagonists and inverse agonists like SR 141716 and CB1 receptor deficient mice have shown that multiple presynaptic cannabinoid receptors are tonically activated by the endogenous cannabinoid ligands. This CB1 receptor mediated inhibition of the transmitter release may be the underlying mechanism for the reinforcing property of cannabis (38).

As mentioned above, THC and other cannabinoids increase dopamine efflux in the nucleus accumbens and pre-frontal cortex and increase dopamine cell firing in the ventral tegmental area. This effect is not caused by direct activation of dopamine neurons because they do not express CB1 receptors. A differential role for endogenous opioid systems as a modulator of cannabinoid

actions in dopamine cell bodies and terminal fields, involvement of glutamatergic and GABAergic inputs to nucleus accumbens and ventral tegmental area, postsynaptic mechanisms involving direct interactions between dopamine D2 receptors and CB1 receptors have been proposed. In agreement with these actions of cannabinoids in brain rewarding circuits, repeated cannabinoid exposure can induce behavioral sensitization similar to other drugs of abuse. Chronic cannabinoid administration also produces cross-sensitization to the locomotor effects of psychostimulants and opioids (39–41).

A number of studies have suggested that there may be links between the development of dependence to cannabinoids and to opiates (42). Some of the behavioral signs of CB1 receptor antagonist, rimonabant (SR141716A)-induced withdrawal, in THC treated rats can be mimicked by administration of the opiate antagonist naloxone (43). Conversely, the withdrawal syndrome precipitated by naloxone in morphine dependent mice can be partly relieved by administration of THC (44) or by endocannabinoids (37). It has also been observed that rats treated chronically with the cannabinoid WIN55,2122 became sensitized to the behavioural effects of heroin (45, 46). Valverde et al. 2001 have demonstrated the anti-nociceptive effects of RB 101, an inhibitor of enkephalin inactivation. These authors found that acute administration of THC caused an increased release of Met-enkephalin into microdialysis probes placed into the rat nucleus accumbens (47). In recent years, the availability of receptor knockout animals has improved our understanding for cannabinoid – opioid interactions. CB1 receptor knockout mice showed reduction in morphine self-administration behaviour and less severe

naloxone-induced withdrawal signs than in wild-type animals, although the anti-nociceptive actions of morphine were unaffected in knockout animals (48). The rimonabant-precipitated withdrawal syndrome in THC-treated mice was significantly inhibited in animals with knockout of the pro-enkephalin gene (47).

Knockout of the  $\mu$  opioid receptor also showed decrease in rimonabant-induced withdrawal signs in THC-treated mice, and attenuated naloxone withdrawal syndrome in morphine dependent CB1 knockout mice (49). These findings clearly points to the interactions between the endogenous cannabinoid and opioid systems in CNS, although the neural circuitry involved is still unclear.

#### **Tolerance, reward and dependence: Preclinical studies**

Tolerance has been defined as the phenomenon of development of decreased response to the previous dose of a drug that leads to an escalation to the amount of the drug needed to produce the same effect. Various animal models have been used to explore the consequences of chronic exposure to the cannabinoid agonists. Recently a number of animal experiments have demonstrated that tolerance develops to most of the behavioural and physiological effects of cannabis (50). Chronic administration of cannabinoids to the animal's results in tolerance to many of the acute effects of  $\Delta^9$ -THC, including memory disruption (18), decreased locomotion (2, 40), hypothermia (24, 50), neuroendocrine effects (51) and analgesia. Single injection of THC (10 mg/kg) has been shown to produce tolerance to the analgesic effect which lasted for a month (12). The most characteristic effect of THC after a single administration

in monkeys is ptosis of the eyelids, docility and loss of aggression. In chronic experiments, tolerance to these effects developed in a few days. They reappeared after the dosage was increased but lasted only for a short period of time. In addition, chronic experiments with rhesus monkeys showed tolerance as well as psychological and physical dependence to THC (23).  $\Delta^9$ -THC does not produce any tolerance to memory deficit in the T maze performance task (52). Several studies have attempted to correlate behavioral tolerance with biochemical alterations, and there is evidence that tolerance to cannabinoids is both pharmacokinetic and pharmacodynamic in nature. Chronic treatment with cannabinoid agonist CP55940 increases the activity of microsomal cytochrome P450 oxidative system suggesting some pharmacokinetic tolerance (18). Chronic cannabinoid treatments also produce changes in brain cannabinoid receptors and cannabinoid receptor mRNA levels, indicating that pharmacodynamic effects are important as well. Effects on CB1 receptor mRNA is not totally consistent, with chronic cannabinoid treatment producing effects on CB1 mRNA levels in the brain (2, 53, 54). The finding that effects on CB1 mRNA levels depend on the time course of treatment and vary between brain regions (55). The development of cannabinoid tolerance is due to pharmacodynamic events and a cross-tolerance among different exogenous cannabinoid agonists has been reported. Animal models have shown the high degree of plasticity that occurs at the molecular level in various brain regions following chronic cannabinoid exposure and the signal transduction pathways (56).

Several studies suggests that brain cannabinoid receptor levels usually decrease

after prolonged exposure to agonists (14, 51, 53, 57), whereas some studies have reported increase (58) or no change (2) in receptor binding in the brain. Evidence of cross-tolerance among various cannabinoid agonists has also been found. Among various possible factors associated with the development of tolerance to the cannabis in animals, studies have been carried out to explore the involvement of role of length of dosing in the onset and maintenance of the tolerance. This study showed that lengths of dosing plays a role not only in the induction of tolerance but also in the degree of tolerance produced. Moreover, earlier time course study also revealed differences in the rates and magnitudes of receptor down-regulation across brain regions (12). These findings suggest that tolerance may develop at different rates to different acute effects of cannabinoids. Furthermore, chronic treatment with  $\Delta^9$ -THC also produces variable effects on cannabinoid-mediated signal transduction systems (14,15, 59).

It is however difficult to extend the findings of the short-term animal studies to human cannabis use as the doses used in animal studies are higher than normally achieved by smoking cannabis in humans. It is likely that some of the same biochemical adaptations to chronic cannabinoid administration occur in both laboratory animals and humans, but the magnitude of the effects in humans may be smaller in proportion to the respective doses used.

Two general procedures that elicit withdrawal responses are abrupt cessation of chronic drug administration and antagonist challenge in animals during chronic drug administration. The onset, intensity, and duration of a withdrawal syndrome are subject to the pharmacokinetic and pharmacodynamic characteristics of the

drug. The sudden termination of chronic treatment with cannabinoids in several different laboratory animal models has not produced uniform findings in animals (13, 34, 43).

There have also been numerous failures in characterizing cannabinoid dependence in rodents. It is probably the long half-life of THC that makes it particularly challenging to observe abrupt withdrawal.

The existence of dependence on cannabinoids in animals has been observed because of the development and availability of selective CB1 receptor antagonist drug SR141716A that can be used to precipitate withdrawal. In contrast to studies confined to employing abrupt withdrawal techniques, SR 141716A has been demonstrated to elicit reproducible and quantifiable withdrawal reactions following chronic cannabinoid administration in variety of laboratory animals (17, 20) demonstrated a behavioral withdrawal syndrome precipitated by SR141716A (rimonabant) in rats treated for only 4 days with doses of THC as low as 0.5-4.0 mg/kg per day. The somatic withdrawal signs included scratching, face rubbing, licking, wet dog shakes, arched back and ptosis. Many of these withdrawal signs are seen in rats undergoing opiate withdrawal. Similar withdrawal signs could be elicited by SR141716A in rats treated chronically with synthetic cannabinoids CP-55, 940 (60) or WIN55, 2122 (3). Although it is clear those SR141716A-precipitated measurable withdrawal responses following THC and other potent cannabinoid analogs, the results with anandamide are less definitive. SR141716A failed to precipitate withdrawal in rats that were infused constantly with anandamide (25–100 mg/kg/day) for 4 days (4). Thus, the potential of anandamide to induce physical dependence remains more of

a controversy and has not been confirmed or refuted beyond doubt. Future studies should be carried out in order to assess whether re-administration of anandamide will reverse these withdrawal signs. Mice lacking FAAH, the primary enzyme responsible for anandamide metabolism, represent another important model for investigating the role of anandamide in cannabinoid dependence (21).

The other important characteristic of a dependence-producing substance is that animals can be trained to self-administer these compounds. It has also been observed that it has been quite difficult to train animals to self-administer cannabinoids as compared to other drugs. Although the physical characteristics of cannabinoids probably contributed too this difficulty, therefore it was thought that cannabinoids lack rewarding effects and therefore are devoid of dependence liability. Persistent intravenous self-administration of synthetic cannabinoid CB1 receptor agonists by rats and mice and the development of genetically modified knocked out mice lacking specific cannabinoid receptors provide convenient models for exploring underlying neurochemical mechanisms. THC and synthetic CB1 agonists can induce conditioned place preferences or aversions, can reduce the threshold for intracranial self-stimulation behavior under certain conditions, and can serve as effective discriminative stimuli for operant behavior provide less direct measures for investigating the rewarding effects of cannabinoids. However, a recent study demonstrated that the synthetic cannabinoid agonist WIN 55, 212–2 may elicit both rewarding and aversive effects depending on the concentration used (61). It is possible that these dual properties have hindered the development of a THC model

of self-administration. Alternatively, decreased response rates at higher doses may be due to the animal reaching a point of satisfaction with total drug intake at lower response rates when higher concentrations are self-administered. As mentioned, the possible substrate for the rewarding properties of the cannabinoids seems to be the mesolimbic dopaminergic system (62). In any case, these studies clearly demonstrate that cannabinoid self-administration is not confined to humans.

#### **Tolerance and dependence: Clinical studies**

Tolerance to cannabis has long been suspected to occur during its continued use. Gross tolerance to the major effects of the cannabis has not been reported, especially with the moderate and intermittent doses. There is very less evidence of tolerance in humans resulting from intermittent use, which may develop after prolonged high doses (7). In a recent epidemiological study the most frequently reported dependence criteria among all users were withdrawal and tolerance reported by 17% and 15% of the respondents respectively (63). THC has effects which in humans somewhat resemble those of hallucinogens and strongly resemble those of alcohol, while in animals it slightly resembles morphine (7). Though it has been argued that many subtle aspects of this topic still needs to be studied in detail. In humans, tolerance only develops with high sustained and prolonged use of the drug. It has also been suggested that  $\Delta^9$ -THC induces drug dependence and tolerance but its action is weaker than those of other drugs of abuse in humans and animals (64). Moreover the phenomenon of reverse tolerance has also been observed following an initial use of cannabis. The underlying mechanism for this phenomenon remain to be poorly understood, this may be because of initial poor smoking

technique, some learning, conditioning or psychological adaptation process, the induction of the enzyme system, accumulation of cannabinoids in the body, receptor sensitization, or more than one of these factors occurring together. It has also been observed that regular users of cannabis learn to control some of the psychological and behavioral effects of cannabis. These abilities may manifest in form of better performance on the cognitive tasks as compared to the same dose exposure given to the non-users, while experiencing the subjective 'high'. This gives the probability of existence of some sort of differential selective adaptation, which may be more of behavioral adaptation to initial disturbing effects rather than being a physical tolerance. This hypothesis is supported by the observation of the similar phenomenon in the subjects after long periods of abstinence. The studies on this phenomenon are marred by the dearth of the available literature. The available human studies have demonstrated a gradual increase in the amount of the cannabis consumed over time associated with some changes in the subjective and behavioral effects (65). Other studies have demonstrated adaptation in the cognitive functions with the daily standard dose use of cannabis (27). On the contrary, other studies have failed to demonstrate any such tolerance on the physiological and psychological parameters (7). The issue of the tolerance to the rewarding and the reinforcing effects of the substance, which may underlie the increased self-administration, cannot be clearly clarified with the available data. The available studies indicate that the tolerance to the cannabis demonstrated by increased dose or frequency of use cannabis is not a prominent characteristic of marijuana under laboratory conditions though it may be observed in

other settings (66). It has been observed that in laboratory conditions it is difficult to give more than 10–15 mg of THC to the light users, without inducing some untoward effects.

Reports from both animals and human do indicate that physical dependence can be induced by abuse of THC. Symptoms of dependence and withdrawal after the frequent administration of high doses (210 mg/kg) of oral  $\Delta^9$ -THC have been reported, yet little is known about dependence on lower oral THC doses (34, 35). Cannabis produces lack of physical dependence and thereby no abstinence syndrome after the discontinuation of the drug. The withdrawal syndrome is unclear, not demonstrated, but may resemble that produced by low doses of opiates, alcohol and other sedatives when they have been given for relatively short periods of time (67). These symptoms may be dose related. Irritability and increased aggression may be associated with withdrawal in chronic cannabis users. Acute abstinence syndrome observed in these cases has been described as the development of irritability, anorexia, insomnia, sweating, headaches and gastrointestinal upset. While physical dependence in case of cannabis remains a controversial issue the development of psychological dependence is even far more elusive concept. There is lack of consensus on the measures of psychological dependence-whether to use drug self-administration paradigm or to go for the concept of acute behavioral withdrawal symptoms, namely anxiety, restlessness, irritability. Presence of daily use of cannabis, associated socio-occupational disruption as observed with other drugs of dependence potential are indicative of development of potential psychological dependence. In comparative studies involving cannabis and tobacco, individuals were more comfortable with the idea of quitting

cannabis use for a day or two in preference to tobacco feeling that they can do better without it rather than the later. These findings raise a doubt over the concept of psychological dependence on cannabis.

Swift et al. (1998) have reported on dependence in cohorts of long-term cannabis users. They reported limited withdrawal phenomenon but argued that this may be related to the sample's ready access to cannabis on a continuous basis. Moreover, they reported that almost one-third of regular cannabis users fell within the definitions of 'substance abuse' (10.7%) or 'substance dependence' (21%). Large-scale population studies report significant rates of cannabis dependence (68) particularly in prison and homeless populations. Scutz et al. (1994) reported the results obtained from a large scale survey which indicated that some 46 % of those interviewed had ever used cannabis and 9% of users became dependent (69). More carefully controlled studies have also demonstrated that a reliable and clinically significant withdrawal syndrome does occur in human cannabis users when the drug is withdrawn. The symptoms include craving for cannabis, decreased appetite, sleep difficulty and weight loss, and may sometimes be accompanied by anger, aggression, increased irritability, restlessness and strange dreams (16). Further studies are required to examine the prevalence and consequences of dependence on cannabis. Furthermore, there is a need for better delineation of the clinical features of cannabis dependence and for studies of its responsiveness to interventions aimed to assisting users to stop.

#### **Commonalities between cannabis and other drugs**

Cannabinoids share a final common neuronal action with the major drugs of

abuse such as morphine, ethanol, amphetamine and nicotine in producing facilitation of the mesolimbic dopamine system (9, 16). In common with other addictive drugs cannabis activates the release of dopamine in the brain. Dopamine release forms part of the brain's reward mechanism, which is involved in dependence. Some regular cannabis users become psychologically dependent and can experience a variety of withdrawal symptoms when they stop using. Although cannabis has traditionally been thought to be different from the drugs of high abuse potential like cocaine and heroin, studies have revealed that not only there is a similarity between cannabis and hard drugs like heroin and cocaine in the neurological effects but also there seems to be a causal link between cannabis use and addiction to the hard drugs (70). Studies have also demonstrated disturbing similarities between 'cannabis' effects on the brain and hard drugs. But these studies have been questioned for their methodological fallacies and the interpretation of the results. The study carried out in Spain concluded that THC tends to be equivalent to heroin in terms of their ability to raise the extracellular concentration of dopamine in the areas of nucleus accumbens and thus can lead on to heroin addiction (71). On the contrary the research in this regard has concluded that the areas in which cannabis has been made more easily available as a result of decriminalization, has not served as a stepping stone for other hard drugs. It has been postulated that cannabis may be serving as a gateway 'away' rather than 'to' the hard drugs. Other differences from hard drugs include that only a minority of those who have used cannabis at some point of time during their life become cannabis addicts and majority of current cannabis users are not addicts.

Cannabis and alcohol have been found to activate the same reward pathways. Moreover, CB<sub>1</sub> receptor system plays an important role in regulating the reinforcing properties of alcohol. Ethanol preference has also been found to be dependent on the cannabinoid receptors and has been substantiated in animal studies (16). Cannabis is also known to act on the opioid system of the brain, which may explain its properties in common with morphine and heroin, for example pain-relief.

#### Future Trends

The discovery of endocannabinoids and the availability of new pharmacological tools, together with the development of strains of genetically engineered knockout mice that lack functional cannabinoid receptors, have revitalized the field of cannabis research in the past few years. The establishment of integrated research using molecular, biochemical, anatomical, and behavioral approaches has made possible to understand the biological basis of cannabinoid dependence. Initial studies in this regard demonstrated the importance of using a brain regional approach to investigate cellular adaptations in response to chronic cannabinoid exposure. Exciting developments in this area have demonstrated that prolonged administration of THC and other cannabinoid agonists produces both desensitization and down regulation of CB<sub>1</sub> receptors in brain, but in a highly region-dependent manner. However, some evidence suggests that these adaptive responses may contribute to the development of cannabinoid tolerance rather than dependence. The discovery of elevated cAMP synthesis and consequent increases in PKA activity in some brain regions during SR 141716-A precipitated withdrawal has led to the

hypothesis that adaptive cellular changes associated with cannabinoid dependence occur downstream of the CB1 receptor-G-protein interaction. Indeed, the ability of chemical modulators of cAMP signaling to block or mimic the expression of cannabinoid withdrawal symptoms give support to this hypothesis. Additional evidence points to upregulation of the stress-activated CRF system in cannabinoid withdrawal along with decreased mesolimbic dopaminergic neurotransmission, suggesting commonality between cannabinoid dependence and

that of other psychoactive drugs. The implementation of genomic approaches, such as DNA arrays that screen thousands of genes simultaneously, will increase our understanding of how cannabinoids alter gene expression in the brain. This approach will undoubtedly lead to new directions in cannabinoid dependence research. Finally, the use of transgenic animal models, such as targeted gene knockouts, will provide a powerful approach to determine the contribution of individual proteins to the process of cannabinoid dependence.

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