CANNABINOID RECEPTORS IN BRAIN: PHARMACOGENETICS, NEUROPHARMACOLOGY, NEUROTOXICOLOGY, AND POTENTIAL THERAPEUTIC APPLICATIONS

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Much progress has been achieved in cannabinoid research. A major breakthrough in marijuana-cannabinoid research has been the discovery of a previously unknown but elaborate endogenous endocannabinoid system (ECS), complete with endocannabinoids and enzymes for their biosynthesis and degradation with genes encoding two distinct cannabinoid (CB1 and CB2) receptors (CBRs) that are activated by endocannabinoids, cannabinoids, and marijuana use. Physical and genetic localization of the CBR genes *CNR1* and *CNR2* have been mapped to chromosome 6 and 1, respectively. A number of variations in CBR genes have been associated with human disorders including osteoporosis, attention deficit hyperactivity disorder (ADHD), posttraumatic stress disorder (PTSD), drug dependency, obesity, and depression. Other family of lipid receptors including vanilloid (VR1) and lysophosphatidic acid (LPA) receptors appear to be related to the CBRs at the phylogenetic level. The ubiquitous abundance and differential distribution of the ECS in the human body and brain along with the coupling to many signal transduction pathways may explain the effects in most biological system and the myriad behavioral effects associated with smoking marijuana. The neuropharmacological and neuroprotective features of phytocannabinoids

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and endocannabinoid associated neurogenesis have revealed roles for the use of cannabinoids in neurodegenerative pathologies with less neurotoxicity. The remarkable progress in understanding the biological actions of marijuana and cannabinoids have provided much richer results than previously appreciated cannabinoid genomics and raised a number of critical issues on the molecular mechanisms of cannabinoid induced behavioral and biochemical alterations. These advances will allow specific therapeutic targeting of the different components of the ECS in health and disease. This review focuses on these recent advances in cannabinoid genomics and the surprising new fundamental roles that the ECS plays in the retrograde signaling associated with cannabinoid inhibition of neurotransmitter release to the genetic basis of the effects of marijuana use and pharmacotherpeutic applications and limitations. Much evidence is provided for the complex *CNR1* and *CNR2* gene structures and their associated regulatory elements. Thus, understanding the ECS in the human body and brain will contribute to elucidating this natural regulatory mechanism in health and disease.

I. Introduction

This review presents the remarkable new understanding that the cellular, biochemical, and behavioral responses to marijuana, which remains one of the most widely used and abused drugs in the world, are coded in our genes. The discovery that specific gene codes for cannabinoid receptors (CBRs) that are activated by marijuana use, and that the human body makes its own marijuana-like substances—endocannabinoids (Onaivi *et al.*, 1996, 2006b)—that also activates CBRs have provided surprising new knowledge about cannabinoid genomic and proteomic profiles. These remarkable advances in understanding the biological actions of marijuana, cannabinoids, and endocannabinoids, is unraveling the genetic basis of marijuana use and the implication in human health and disease. In the era of personalized medicine, we are eager to understand how to utilize cannabinoid pharmacotherapeutic agents based on our new knowledge of the genetic basis of cannabinoid and endocannabinoid action. We know that the two well-characterized cannabinoid CB1 and CB2 receptors are encoded by *CNR1* and *CNR2* genes that have been mapped to human chromosome 6 and 1, respectively. A number of variations in CBR genes have been associated with human disorders including osteoporosis (Karsak *et al.*, 2005; Sipe *et al.*, 2005), ADHD (Lu *et al.*, 2008), posttraumatic stress disorder (PTSD) (Lu *et al.*, 2008), drug dependency (Onaivi *et al.*, 2006b), obesity (Cota *et al.*, 2003; Jesudason and Wittert, 2008), and depression (Onaivi *et al.*, 2006a; Serra and Fratta, 2007).

Thus, because of the ubiquitous distribution and role of the endocannabinoid system in the regulation of a variety of normal human physiology, drugs that are targeted to different aspects of this system are already benefiting cancer subjects and those with AIDs and metabolic syndromes (Jesudason and Wittert, 2008). In the coming era of personalized medicine, genetic variants and haplotypes in *CNR1* and *CNR2* genes associated with obesity or addiction phenotypes may help identify specific targets in conditions of endocannabinoid dysfunction.

Our previous investigations had defined a number of features of the *CNR1* gene's structure, regulation, and variation (Zhang *et al.*, 2004), but many of these features still remain poorly defined. Nevertheless, we and others have now demonstrated and reported that variants of the *CNR1* gene are associated with a number of disorders and substance abuse vulnerability in diverse ethnic groups including European–American, African–American, and Japanese subjects (Zhang *et al.*, 2004). Most strikingly, variants of *CNR* genes co-occur with other genetic variations and share biological susceptibility that underlies comorbidity in many neuropsychiatric disturbances (Palomo *et al.*, 2007). Thus, emerging evidence indicates that the endocannabinoid system exerts a powerful modulatory action on retrograde signaling associated with inhibition of synaptic transmission (Lovinger, 2008; Onaivi *et al.*, 2006b). Interestingly, a role for variations in *CNR1* gene has been associated with striatal responses to happy, but not to disgust, faces (Chakrabarti *et al.*, 2006) with implication that functional variation of *CNR* genotypes may be associated with disturbances of the brain involving emotional and social stimuli, such as autism (Chakrabarti *et al.*, 2006) and depression (Domschke *et al.*, 2008; Onaivi *et al.*, 2008a). This review focuses on these recent advances in cannabinoid genomics and the surprising new fundamental roles that the endogenous endocannabinoid system (ECS) plays in the genetic basis of the effects of marijuana use. The powerful influence of cannabinoid-induced retrograde signaling modulates GABAergic and glutamatergic systems indicate that the main excitatory and inhibitory systems are in part under the influence of the endocannabinoid system. Additional evidence is provided for the complex *CNR1* and *CNR2* gene structures and their associated regulatory elements. Therefore, understanding the ECS in the human body and brain will contribute to elucidating this natural regulatory mechanism in health and disease.

II. Cannabinoid Genomics

Most of the physiological effects of smoking marijuana are probably mediated via CBRs, actions at other non-CBRs, and nonreceptor mechanism(s). Thus, the genetic basis of marijuana use and perhaps dependence may be associated at least in part to their effects on these G-protein-coupled CBRs. The presently known

CBRs and their gene transcripts can now be analyzed in human blood samples (Onaivi *et al.*, 1999), fetal brain (Mato *et al.*, 2003; Wang *et al.*, 2003), placenta (Park *et al.*, 2003), uterus during pregnancy (Dennedy *et al.*, 2004), Alzheimer's disease brains (Benito *et al.*, 2003), prefrontal cortex of depressed suicide victims (Hungund *et al.*, 2004), cognitive impairment in multiple sclerosis (Woolmore *et al.*, 2008), substance abuse and dependence (Herman *et al.*, 2006; Schmidt *et al.*, 2002; Zhang *et al.*, 2004; Zuo *et al.*, 2007), and obesity phenotypes (Russo *et al.*, 2007). The human CB1-R cDNA was isolated by Gerard *et al.* (1991) from a human brain stem cDNA library using a 600 bp DNA probe and polymerase chain reaction. The deduced amino acid sequences of the rat and human receptors showed that they encode protein residues of 473 and 472 amino acids with 97.3% homology. These proteins share the seven hydrophobic transmembrane domains and residues common among the family of G-protein receptors (Gerard *et al.*, 1991; Matsuda *et al.*, 1990; Shire *et al.*, 1995) (Table I). The human (Fig. 1) and rat CB1-Rs also share pharmacological characteristics including the inhibition of adenylate cyclase activity via Gi/o in a stereoselective and pertusis sensitive manner following activation by cannabinoids. The CB1-Rs alter potassium channel conductance and decrease calcium channel conductance. The expression of the CB1-Rs is particularly dense in presynaptic terminals and endocannabinoids act as retrograde messengers at many synapses in the CNS. Although, the transcripts for both CB1 and CB2 receptors (Figs. 1 and 2) were reported to be present in the human placenta, the identification of CB1-Rs and fatty acid amide hydrolase in the human placenta may be targets for the effects marijuana use during pregnancy (Park *et al.*, 2003). Most of the studies, examining the expression and role of CBRs in the reproductive system have used mice where both CB1 and CB2 receptor gene transcripts were identified in mouse preimplantation embryos (Battista *et al.*, 2008). There is a close link between the endocannabinoid system and sex hormones in male and female reproductive system (Battista *et al.*, 2008). From mouse model to humans, the CBRs are a placental and uterine site of action of endocannabinoids and marijuana use during pregnancy and parturition. The identification of CBRs in the uterus, placenta, and the high densities of CB1-Rs, functionally coupled to G-protein in prenatal developmental stages throughout the human brain, suggest the involvement of the cannabinoid system in neural development (Mato *et al.*, 2003). It has also been suggested that the high expression of CB1 mRNA in the human fetal limbic structures may render such brain structures more vulnerable to prenatal cannabis exposure (Wang *et al.*, 2003). In adult diseased brains, an upregulation of CB1-Rs and agonist-stimulated [^{35}S] GTPγS binding in the prefrontal cortex of diseased suicide victims was reported in comparison to matched controls (Hungund *et al.*, 2004). This and other observations of the involvement of the ECS in a variety of CNS disorders renders different component of the ECS possible targets in the treatment of a variety of CNS mental and neurological disturbances. A trinucleotide repeat (AAT)

TABLE I
SUBTYPES OF CANNABINOID RECEPTORS

Hydrophobic transmembrane domains	CB1 Receptors	CB2 Receptors
Amino acids	472 AA	360 AA
Chr Location	6q14–15	1p36.11
Gene name	*CNR1*	*CNR2*
Endogenous ligand	2-AG	2-AG
CNS distribution	Yes	Yes
Peripheral distribution	Yes	Yes
Subtypes[a]	CB1 and CB1A-CB1n	CB2A and CB2B

[a]See text for isoforms and variants of CB1 and CB2 receptors.

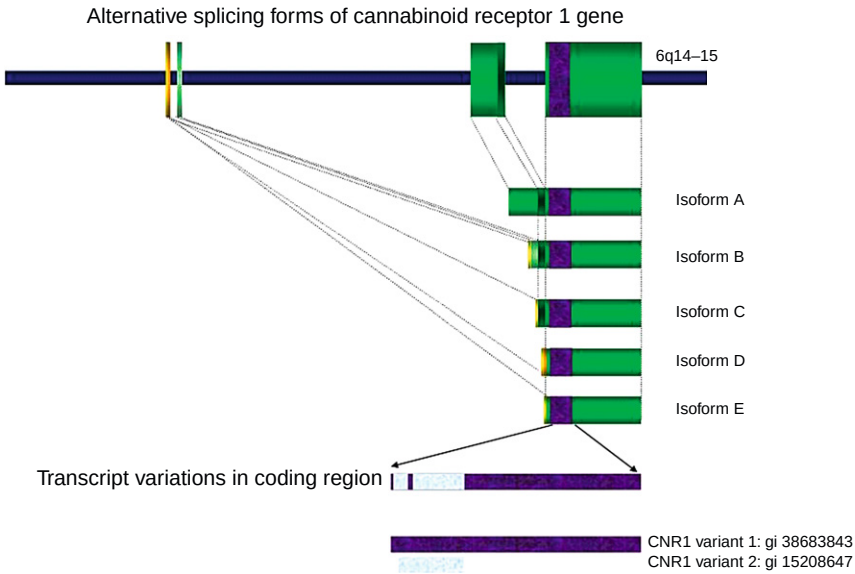

FIG. 1. Structure of CB1 *CNR* gene. Genetic structure of the CB1-R *CNR* gene. The gene is mapped on two finished genomic sequences: The complete mRNA sequence and transcript size is known. The sequences consist of exons, one of them include the whole coding region of the gene. A number of ESTs are identified and surprisingly some of them are between the exons of the published mRNA. A number of polymorphisms have been identified in the CB1 *CNR* gene and discussed.

polymorphism of the *CNR1* gene has been associated with appetite disorder involving the binging/purging type of anorexia nervosa, which is a severe and disabling psychiatric disorder characterized by profound weight loss and body image disturbance (Siegfried *et al.*, 2004).

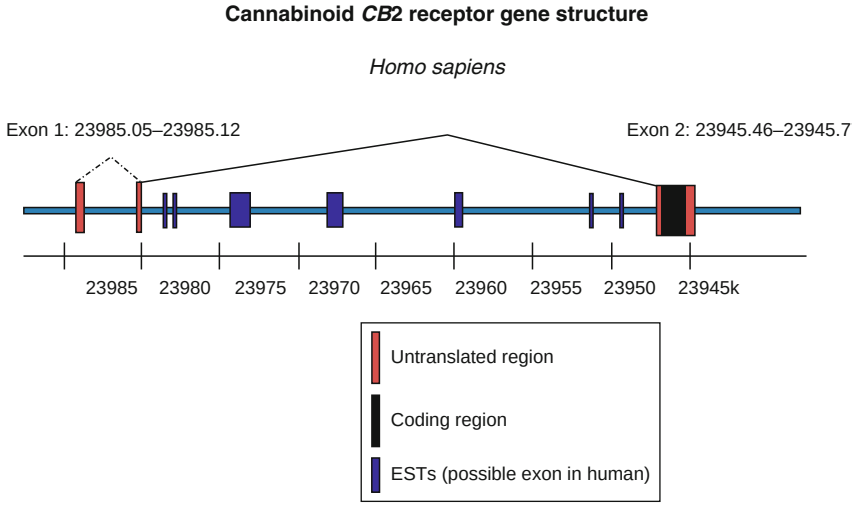

FIG. 2. Structure of CB2 *CNR* gene. Genetic structure of the CB2-R *CNR* gene. Some variants have been identified and some known polymorphisms in the CB2 *CNR* gene are discussed.

The assembly of the human CB1-Rs and the impact of its long N-terminal tail has been examined by Andersson *et al.* (2003). They found that the long N-tail of CB1-Rs that lack a typical signal sequence and cannot be efficiently translocated across endoplasmic reticulum membrane might not be relevant to ligand binding or activation of the receptor (Andersson *et al.*, 2003). With the sheer abundance of CBRs, the functional significance of the existence of a CB1-R with long N-terminal and a shorter N-terminal tail in CB1A receptor are not immediately clear, but may be related to rapid formation of different tonically active states allowing for sequestration of G-proteins, making them unavailable to couple to other receptors (Vasquez and Lewis, 1999). One of the major advantages for the physiological actions of these CBRs may be associated with the quick response needed for the sequestration of G-proteins and the retrograde signaling of endocannabinoids on presynaptic CB1-Rs to inhibit neurotransmitter release. The second cannabinoid CB2-R clone was isolated from myeloid cells by PCR and degenerate primers using cDNA template from human promyelocytic leukaemic line HL60 (Munro *et al.*, 1993). Similar hydrophobic domains 1, 2, 5, 6, and 7, in which 50% or greater of the amino acids were identical between CB1-Rs and CB2-Rs. The extracellular domain also contained sequence motifs that were common to both clones (Matsuda, 1997). The protein encoded by the *CNR2* gene shows 44% identity with the human *CNR1* gene (Table I). A number of functional and expression studies have been performed with the *CNR2* gene and the results indicate that the CB2-R is the predominant CBR in the immune

system, where it is expressed in B and T cells (Gurwitz and Kloog, 1998; Munro *et al.*, 1993; Schatz *et al.*, 1997). A number of laboratories were not able to detect the presence of CB2-Rs in healthy brains, but there had been demonstration of CB2-R expression in rat microglial cells and other brain associated cells during inflammation (Onaivi *et al.*, 2006b). We and others have now identified and reported the presence of CB2-Rs in brain neuronal and glial process (Beltramo *et al.*, 2006; Gong *et al.*, 2006; Onaivi *et al.*, 2006b, Van Sickle *et al.*, 2005). To further improve understanding of the role of CB2-Rs in the brain, we hypothesized that genetic variants of *CNR2* gene might be associated with depression in a human population and that alteration in *CNR2* gene expression may be involved in the effects of abused substances in rodents. Our data reveals that CB2-Rs are expressed in brain and plays a role in depression and substance abuse (Onaivi *et al.*, 2008b). The relevance of CB2-Rs, if any to problem and compulsive marijuana use is unknown. However, a specific enzyme activity by a phosphodiesterase of the phospholipase D-type responsible for generating endolipids like anandamide has been identified, cloned from mouse, rat and human and characterized, with the expression of the mRNA, and proteins widely distributed in murine brain, kidney, and testis (Okamoto *et al.*, 2004). The direct contribution of this enzyme and its influence in the effects of abused substances and marijuana dependence has not been determined, but the levels of *N*-acylethanolamines are known to increase in a variety of animal models of tissue degeneration (Okamoto *et al.*, 2004). Furthermore, cells and tissues involved in neuroinflammation express functional CBRs with an upregulation of components of the endocannabinoid signaling system in the inflammatory response (Walter and Stella, 2004). As the cannabinoid signaling machinery is involved in immune function, it is yet unknown if neuroinflammatory processes contribute to continued marijuana use and dependence, once initiated. Other, as yet unknown, components of the cannabinoid system have been shown to be involved in neuroinflammatory processes that are sensitive to the nonpsychotropic CBRs whose molecular identity is currently unknown. This additional evidence about cannabinoid regulation of neuroinflammation and immunomodulation has been provided by 2-AG stimulation of microglial cell migration that is blocked by nonpsychotropic CBR antagonists, cannabinoid, and abnormal cannabidiol (Walter and Stella, 2004). It appears therefore that the vital role played by cannabinoids system in the neuroinflammation and immunomodulation may be exploited in the development of immunopharmactherapy against drugs of abuse by targeting some component of the ECS. As there is accumulating evidence indicating a central role for the endocannabinoid system in the regulation of the rewarding effects of abused substances, an endocannabinoid hypothesis of drug reward and addiction has been postulated (Onaivi *et al.*, 2008). In humans, the genetic liability following fetal exposure to marijuana during pregnancy might be a predisposing vulnerability factor to use marijuana in adulthood. Earlier studies have reported on the

capability of marijuana use to induce genotoxity (see Li and Lin, 1998, for a review). Many other studies have shown that the allele frequency distributions of many genetic variants at *CNR1* gene differ by population and sex (Zhang *et al.*, 2004; Zuo *et al.*, 2007).

III. Cannabinoid Neuropharmacology, Neurogenesis, Neurotoxicology, and Neuroprotective Properties

The neuropharmacological and neuroprotective features of phytocannabinoids (de Lago and Fernandez-Ruiz, 2007) and endocannabinoid associated neurogenesis (Goncalves *et al.*, 2008) have revealed roles for the use of cannabinoids in neurodegenerative pathologies with less neurotoxicity. A role for CB2-Rs and not for CB1-Rs has been reported in the regulation of adult subventricular zone neurogenesis in an age-dependent manner (Goncalves *et al.*, 2008). Acute toxicity studies show marijuana use or acute administration of THC, the main psychoactive ingredient of cannabis does not lead to death (Beaulieu, 2005). In deed unlike the opiate receptors, CBRs appear to be sparsely distributed in areas of the brain that lead to respiratory depression in opiate overdose causing death. It has been demonstrated that in traumatic brain injury, neuroprotection is exerted by the release of 2-AG that may serve as a molecular regulator of pathophysiological events in reducing brain damage (Mechoulam and Shohami, 2007). There is also growing evidence that the endocannabinoids are lipid mediators that are involved in the control of neuron survival (Galve-Roperh *et al.*, 2008). Therefore, different mechanisms have been associated with CBRs and their role in neuroprotection (Van Der Stelt and Di Marzo, 2005).

IV. Chromosomal Mapping of the *CNR* Genes

The two well-documented human CBR *CNR* genes and of many other mammalian species have been mapped to their respective chromosomes. Human *CNR1* and *CNR2* genes have been mapped to chromosome 6q14-q15 and 1p34-p35, respectively. Using genetic linkage mapping and chromosomal *in situ* hybridization the genomic location of the human *CNR* gene was determined, thus confirming the linkage analysis and defining a precise alignment of the genetic and cytogenetic maps (Hoehe *et al.*, 1991). These investigators found that the location of the human CB1 *CNR* gene is very near the gene encoding the alpha subunit of chorionic gonadotropin (CGA). After we cloned and sequenced the mouse CB1 *CNR* gene (Chakrabarti *et al.*, 1995), we determined that the

mouse CB1 and CB2 *CNR* genes are located in proximal chromosome 4 (Stubbs *et al.*, 1996). This location is within a region to which other homologs of human 6q genes are located. The CB1 *CNR* gene, GABRR1, GABRR2, and Cga are linked together both in the mouse chromosome 4 and on human chromosome 6q (Fig. 3). The results of the chromosomal location of the human *CNR* gene (Hoehe *et al.*, 1991) and the mouse (Stubbs *et al.*, 1996) *CNR* genes add a new marker to this region of the mouse–human homology, and confirm the close linkage of *CNR* genes in both species Fig. 3. Further, the location of the rat CB1 *CNR* gene in the rat genome has been determined and fits the rodent–human homology as the CB1 *CNR* genes are highly conserved in the mammalian species. The genomic sequences (using complete or draft) of a part of chromosome 6p covers more than 200kb around the CB1/CNR gene and 90 Kb around the human *CNR2* gene. The program GENESCAN was used to estimate four fairly possible gene sequences including CB1 *CNR* gene in this region. These estimated sequences showed similarity to known protein sequences, revealed by BLAST. As the neurobiological effects of marijuana and other cannabinoids suggest the involvement of the *CNR* genes in mental and neurological disturbances, the mapping of the genes will undoubtedly enhance our understanding of the linkage and possible cannabinoid genetic abnormalities. The chromosomal location and genomic structure of human and mouse fatty acid amide hydrolase (FAAH) genes

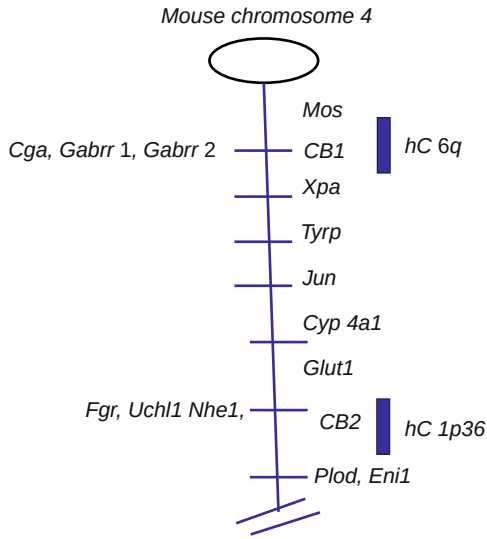

FIG. 3. Chromosomal localization of CB1-R and CB2-R *CNR* genes. The chromosomal locations of CB1-R and CB2-R CNR genes are on mouse chromosome 4 and human chromosome 6 and 1P36, respectively.

have been mapped to chromosomes 1p34-p35 and 4 (Wan *et al.*, 1998). The localization of FAAH and CB2 *CNR* genes are in same chromosomal regions in the mouse and human and again adds a new marker to this region of the mouse–human homology, and confirms the close linkage of FAAH and *CNR* genes in both species.

V. Polymorphic Structure of *CNR* Genes

Although two CBR G-protein coupled receptor (GPCR) subtypes have been cloned and well characterized, studies now indicate the functional existence of splice variations of CB1- and CB2-Rs. New information about *CNR* genes and their allelic variants in humans and rodents is adding to our understanding of vulnerabilities to addictions and other neuropsychiatric disorders and the genetic basis of marijuana use and addiction in vulnerable individuals. Some of the complexities are however due to our lack of understanding of the regulation of the CBR genes and the molecular identity of other subtypes (if any). The known and cloned CBRs are designated as *CNR1* and *CNR2* or CB1-R and CB2-R. They belong to the large super family of receptors that couple to guanine–nucleotide–binding proteins and that thread through cell membranes seven times (heptahelical receptors). The CB1-R is predominantly expressed in brain and spinal cord, and thus often referred to as the brain CBR. A spliced variant of human CB1-R, cDNA, CB1A, was isolated and characterized (Rinaldi-Carmona *et al.*, 1996; Shire *et al.*, 1995). It appears that the CB1A resulted from the excision of an intron at the 5′-extremity of the coding region of the human receptor mRNA (Shire *et al.*, 1995). This subtype of CBRs were stably expressed in Chinese hamster ovary cell lines and the truncated and modified CB1 isoform CB1A was shown to exhibit all the pharmacological properties of CB1-R to a slightly attenuated extent (Rinaldi-Carmona *et al.*, 1996). A study of the distribution of the human CB1A mRNA by reverse transcriptase PCR showed the presence of minor quantities of this isozyme together with CB1-R throughout the brain and in all the peripheral tissues examined (Shire *et al.*, 1995). While the presence of CB1A has not been detected in rodents, evidence for the existence of unknown CBR subtypes in mouse brain has been described (Onaivi *et al.*, 2006b). The CB2-R is, at times, referred to as the peripheral CBR because of its largely peripheral expression in immune cells. Results of new studies indicate that CB2-Rs are expressed in the neuron and glial cells in the brain particularly during pathological events (Benito *et al.*, 2003) and in mast cells (Samson *et al.*, 2003). CB1 and CB2 receptor gene products are expressed in relative abundance in specific tissues and cell types. CB1-Rs are considered to be the most abundant GPCRs in the mammalian brain (Matyas *et al.*, 2006; Onaivi *et al.*, 2002a). These CB1-Rs are

highly expressed in brain areas such as the cortex, limbic system, hippocampus, cerebellum, and several nuclei of the basal ganglia; areas associated with emotionality, cognition, motor, and executive function. CB2-Rs on the other hand were known to be expressed in peripheral and immune tissues and have traditionally been referred to as peripheral CB2-Rs, because a number of previous studies did not detect the presence of CB2-Rs in the mammalian brain (Brusco *et al.*, 2008a). Although glial expression of CB2-Rs was to be expected and have been reported by many investigators (Brusco *et al.*, 2008b), the functional expression of neuronal of CB2-Rs had been controversial. We and others have now identified and reported the presence of CB2-Rs in brain neuronal cells (Ashton *et al.*, 2005; Gong *et al.*, 2006; Jhaveri *et al.*, 2008; Onaivi *et al.*, 2006a; Van Sickle *et al.*, 2005) and this warrants a re-evaluation of the role of CB2-Rs in the brain. With novel and precise cannabinoid probes, our results indicate the expression of brain CB2-Rs in a mouse model of depression and in the effects of abused substances (Onaivi *et al.*, 2008a).

This review discusses the current state of description of the genes encoding CBRs and examines the genetic basis of marijuana use; and also defines some of the limitations of current knowledge. The molecular biology and pharmacology of the CBRs is still less well understood than that of many GPCRs. Only scanty information describes how these CBR *CNR* genes are regulated. A moderate store of data describes some of the complex signal transduction pathways engaged by CBR activation (Onaivi *et al.*, 2002b). However, many topics including the ways in which the abundant CB1-Rs could alter activities mediated through other co expressed GPCRs by sequestering G-proteins and other means are still been investigated. With regards to these GPCR-CBRs, we also need to be aware of the possible ligand gated ion channels influenced by cannabinoids and interactions at the vanilloid receptors. We need to bear in mind data suggesting that cannabinoids can exert receptor-independent effects on biological (Hillard *et al.*, 1985; Makriyannis *et al.*, 1989) and enzyme systems such as protein kinase C. Despite these caveats, it is impressive to consider the large amount of data about CBRs amassed over the last decade, which has enhanced our knowledge of the physiological factors that triggers and contributes to the cycle of marijuana use.

There is increasing information and knowledge available at the molecular level about *CNR* gene structure, regulation and polymorphisms. Different human *CNR* gene polymorphisms have been reported (Table II). These polymorphic sequences are either single nucleotide polymorphisms (SNPs) or larger changes in the number of repeats. We have identified polymorphisms in nontranslated region that affect splicing and promoter activity. As described in Table II, some of the SNPs are silent to translation, while others lead to amino acid substitutions in the CBR protein. Many of the currently known genetic polymorphisms of the endocannabinoid system have been linked with a number of conditions including substance abuse, mental disorders and energy metabolism and have been

TABLE II
GENETIC POLYMORPHISMS OF CANNABINOID RECEPTOR GENES (*CNR* GENES)

CNR genes	Polymorphism	Linkage or association
CB1	Two allele DNA polymorphism	Associated with *CNR1* gene (Caenazzo *et al.*, 1991)
	1359 G/A CNR1 variant	Associated with alcohol, dependence (Gadzicki *et al.*, 1999; Schmidt *et al.*, 2002)
	1359 G/A CNR1 variant	Not associated with Tourette syndrome (Gadzicki *et al.*, 2004)
	1359 G/A CNR1 variant	Not associated with Alcohol Withdrawal tremens (Preuss *et al.*, 2003)
	1359 G/A CNR1 variant	Associated with weight loss (Aberle *et al.*, 2007, 2008)
	3813 A/G and 4895 A/G variant	Associated with obesity in men (Russo *et al.*, 2007)
	CNR1 SNPs	No association in obesity in German children (Aberle *et al.*, 2007; Muller *et al.*, 2007)
	CNR1 SNPs	Associated with obesity and BMI (Benzinou *et al.*, 2008; Gazzerro *et al.*, 2007; Jaeger *et al.*, 2008; Peeters *et al.*, 2007)
	CNR1, FAAH, DRD2 gene	Associated with comorbidity of alcoholism and antisocial behavior (Hoenicka *et al.*, 2007).
	(AAT)n repeat of CNR1 gene	Conflicting associations with drug dependence (Ballon *et al.*, 2006; Comings, 1996; Comings *et al.*, 1997; Covault *et al.*, 2001; Heller *et al.*, 2001; Jesudason and Wittert, 2008).
	CNR1 variants, SNPs, 'TAG' haplotype	Associated with polysubstance abuse (Zhang *et al.*, 2004; Zuo *et al.*, 2007)
	CNR1 SNPs	Not associated with polysubstance abuse (Herman *et al.*, 2006)
	CNR1 SNPs	Associated with cannabis dependence (Agrawal *et al.*, 2008, 2009)
	CBR haplotype	Associated with fewer cannabis dependence symptoms in adolescents (Hopfer *et al.*, 2003, 2006, 2007).
	CNR1 SNPs	Associated with alcohol and nicotine dependence (Chen *et al.*, 2008; Hutchison *et al.*, 2008)
	CNR1 SNPs	No association with anorexia nervosa (Muller *et al.*, 2008)
	CNR1 (AAT)n repeats	Associated with restricting and binging/purging anorexia nervosa (Siegfried *et al.*, 2004)
	CNR1 (AAT)n repeats	Associated with depression in Parkinson's disease (Barrero *et al.*, 2005)
	CNR1 SNPs	Associated to striatal responses to facial exp. (Chakrabarti *et al.*, 2006)
	(AAT)n repeats	Association with ADHD in alcoholics (Lu *et al.*, 2008; Ponce *et al.*, 2003)

(*continued*)

TABLE II (*continued*)

CNR genes	Polymorphism	Linkage or association
	CNR1 SNP haplotype	Risk factor for ADHD and PTSD (Lu *et al.*, 2008)
	1359 G/A *CNR1* variant	Associated with schizophrenia (Leroy *et al.*, 2001)
	(AAT)n repeats	Not associated with schizophrenia (Li *et al.*, 2000; Tsai *et al.*, 2000) and mood disorders (Tsai *et al.*, 2001)
	(AAT)n repeats	Associated with schizophrenia (Martinez-Gras *et al.*, 2006)
	(AAT)n repeats	Associated with hebephrenic schizophrenia (Chavarria-Siles *et al.*, 2008; Ujike *et al.*, 2002)
	CNR1 variants	Associated with depression and anxiety (Domschke *et al.*, 2008)
	CNR1 variants and (AAT) n repeats	Associated with impulsivity (Ehlers *et al.*, 2007a,b)
	1359 G/A *CNR1* tag SNP	Associated with antipsychotic response but not schizophrenia (Hamdani *et al.*, 2008)
	CNR1 SNPs	No association with cognitive impairment in MS (Woolmore *et al.*, 2008)
CB2	*CNR2* SNPs and haplotypes	Associated with human Osteoporosis (Karsak *et al.*, 2005)
	CNR2 SNPs	Not associated with myocardial infarction or cardiovascular risk factors (Reinhard *et al.*, 2008)
	CNR2 SNPs	Associated with bone mass (Yamada *et al.*, 2007)
	CNR2 (Q63R) SNP	Risk factor for autoimmune disorders (Sipe *et al.*, 2005)
	CNR2 (Q63R) but not (H316Y)	Associated with alcoholism and depression (Ishiguro *et al.*, 2007)

reviewed (Norrod and Puffenbarger, 2007). A silent mutation of a substitution from G to A, at nucleotide position 1359 in codon 453 (Thr) that turned out to be a common polymorphism in the German population was reported (Gadzicki *et al.*, 1999). In this study, allelic frequencies of 1359(G/A) in genomic DNA samples from German Gilles de la Tourette syndrome (GTS) patients and controls were determined by screening the coding exon of the CB1 *CNR* gene using PCR single-stranded conformation polymorphism (PCR-SSCP) analysis (Gadzicki *et al.*, 2004). This was accomplished by the use of a PCR based assay by artificial creation of a MSP1 restriction site in amplified wild-type DNA (G-allele), which is destroyed by A-allele (Gadzicki *et al.*, 2004). They found no significant differences of the allelic distributions between GTS patients and controls within the coding region of the CB1 *CNR* gene. In our studies the frequencies of this polymorphism are significantly different between Caucasian, African–American, and Japanese population (Zhang *et al.*, 2004). There is also a HindIII restriction fragment length polymorphism (RFLP) located in an intron approximately 14 kb

in 5′ region of the initiation codon of the CB1 *CNR* gene. Caenazzo *et al.* (1991) genotyped 96 unrelated Caucasians using hybridization of human DNA digested with HindIII and identified a two allele with bands at 5.5 (A1) and 3.3 kb (A2). The frequencies of these alleles were 0.23 and 0.77, respectively. Another polymorphism is a triplet repeat marker for CB1 *CNR* gene. This is a simple sequence repeat polymorphism (SSRP) consisting of nine alleles containing (AAT) 12–20 repeat sequences that was identified by Dawson (1991). This polymorphism has been used in linkage and association studies of the CB1 *CNR* gene with mental illness and drug abuse in different population. This CB1 *CNR* gene triplet repeat marker was used to test for linkage with schizophrenia using 23 multiplex schizophrenia pedigrees (Dawson, 1991) and association with heroin abuse in a Chinese population (Li *et al.*, 2000) and also for association with intravenous (IV) drug use in Caucasians (Comings *et al.*, 1997). There was no linkage and association of the marker with schizophrenia indicating that the *CNR1* gene is not a polymorphism of major aetiological effect for schizophrenia but *CNR1* gene might be a susceptibility locus in certain individuals with schizophrenia, particularly those whose symptoms are apparently precipitated or exacerbated by cannabis use (Dawson, 1991). Comings *et al.* (1997), hypothesized those genetic variants of CB1 *CNR* gene might be associated with susceptibility to alcohol or drug dependence and analyzed the triplet repeat marker in the CB1 *CNR* gene. They found a significant association of the CB1 *CNR* gene with a number of different types of drug dependence (cocaine, amphetamine, and cannabis), and intravenous drug use but no significant association with variables related to alcohol abuse/dependence in non-Hispanic Caucasians (Table II). In addition, this group also reported that a significant association of the triplet repeat marker in the CB1 *CNR* gene alleles with the P300 event related potential that has have been implicated in substance abuse (Johnson *et al.*, 1997). Li *et al.* (2000) attempted to replicate the finding of Comings *et al.* (1997), in a sample of Chinese heroin addicts and did not find any evidence that the CB1 *CNR* gene AAT repeat polymorphism confers susceptibility to heroin abuse. CB1 *CNR* gene is located in human chromosome 6q14-q15 and it is interesting that previous reports showed evidence for suggestive linkage to schizophrenia with chromosome 6q markers (Martinez *et al.*, 1999) and also suggestive evidence exist for a schizophrenia susceptibility locus on chromosome 6q (Cao *et al.*, 1997). Although there was no linkage and association of the CB1 *CNR* triplet marker with schizophrenia, it remains to be determined if linkage and association to schizophrenia might exist with other unknown polymorphisms that might exist in the CB1 *CNR* gene structure that is currently poorly characterized. Three other variants have been reported in the CB1 *CNR* gene of an epilepsy patient (Kathmann *et al.*, 2000). This was obtained from PCR assay with cDNA from hippocampal tissue taken from patients undergoing neurosurgery for intractable epilepsy. They detected four mutations in the coding region of the CB1 *CNR* gene, with the first three mutations yielding amino acid substitutions.

Our previous studies analyzed CB1 *CNR* gene structure, regulation, and expression in the mouse and human models to determine genotypic and/or haplotypic associations of CB1 *CNR* gene with addictions and other neuropsychiatric disturbances. The human Chr 6, 91.8∼96.1 cM CB1/*CNR1* locus encodes at least six exons which account for 24–28 kb of sequence (Zhang *et al.*, 2004). Examination of CB1 *CNR* gene sequence variations in distinct populations has revealed a G/A SNP in CB1 5′ flanking sequences. The initial values for linkage disequilibrium between these markers and genotypic frequencies of the markers in drug abusing and control populations were calculated. We tested for associations between some SNPs and the risk for polysubstance use disorders in Japanese, European–American, and African–American populations and reported the association between some SNPs in *CNR1* gene and vulnerability to polysubstance use disorder (Zhang *et al.*, 2004). Some other studies have reported similar findings while others have been equivocal (Table II). While polymorphisms in *CNR2* gene have not been well characterized in linkage or association studies in comparison to *CNR1* gene, *CNR2* gene polymorphism has been linked to autoimmune disorders including osteoporosis (Karsak *et al.*, 2005; Sipe *et al.*, 2005) and with bone mineral density in Japanese population (Yamada *et al.*, 2007). We have reported the association between Q63R polymorphism of the CB2 *CNR2* gene and alcoholism (Ishiguro *et al.*, 2007) and depression (Onaivi, 2006) in Japanese population. Another area of research is on the polymorphisms associated with the metabolizing enzymes of the endocannabinods which is not addressed in the review. Thus, polymorphisms at the CBR *CNR* genes may be associated with the diverse actions of marijuana use, and may therefore appear to be a major factor which when triggered by environmental, age, and metabolic factors could lead to the continuous circle of marijuana dependence and use. Cannabinoid research and the use of cannabis products continue to attract significant attention. The current significant advances in molecular biology and technology that increased scientific knowledge in cannabinoid research will certainly contribute to a better policy on the medical use of marijuana. For example, studies with CBR antagonists have contributed to resolving the long-standing debate about addiction to marijuana. Specifically, the controversial question of physical dependence on psychoactive cannabinoids has now been addressed, using the antagonist, rimonabant, also known as SR 141716A [*N*-(piperidin-1-yl)5-(4-chlorophenyl)-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride, to precipitate withdrawal reactions in rats injected with increasing doses of Δ^9-THC. Aceto *et al.* (1996) reported a precipitated withdrawal syndrome that was absent in control animals, providing evidence that Δ^9-THC could produce physical dependence. In general it had been claimed that the psycho activity and euphoria induced by cannabinoids limit their use in the clinic for numerous therapeutic applications for which they are currently being evaluated. With the availability of these genes, gene products and other CBR research tools, it is speculated that the

properties of these genes and regulation will be intensely studied as to reveal how the psycho activity can be dissociated from the therapeutic properties of marijuana and cannabinoids. Or could it be that certain therapeutic actions of marijuana and cannabinoids cannot be separated from their psycho activity?

VI. Genes Encoding Endocannabinoid Transporter(s) as Pharmacotherapeutic Targets

Although there is evidence from functional studies for the existence of some form of cannabinoid transporter(s), their identity, sequence information, and biological characteristics at the molecular level are unknown. This is therefore "a difficult issue to handle," as concluded by the review on anandamide transport (Glaser *et al.*, 2005). Without a molecular identity and with the different experimental approaches in assay conditions that support or refute the existence of a transporter for anandamide, the cloning and identification of genes encoding these endocannabinoid transporter(s) will certainly resolve the issue. While there is ample scientific evidence to support the concept that anandamide transport across membranes is protein mediated, definitive evidence awaits its molecular characterization. It has also been suggested that endocytosis via a caveolae/lipid raft mediates anandamide transport. However, the differential uptake of the different endocannabinoids, for example anandamide and 2-AG in different cell types may indicate the possibility of different cannabinoid transporters for the different endocannabinoids (Glaser *et al.*, 2005). If there is a specific 2-AG membrane transport, only a few studies have addressed the transport of 2-AG. The scanty reports based upon saturation kinetics have proposed that 2-AG enters the cell by a specific 2-AG transporter, via the anandamide transporter or by simple diffusion. It was therefore suggested that the mechanism of 2-AG transport appears to be rate-limited diffusion through the membrane (Hermann *et al.*, 2006). Further research will show whether the additional endocannabinoids been discovered, like noladin ether will be metabolized and taken up by similar metabolic and uptake inhibitors. This will not be unprecedented since the monoamines have different transporters for dopamine, serotonin and norepinephrine. Nevertheless, endocannabinoids may be a distinct class of agonist since they are hydrophobic and neutral, exhibiting similar biophysical properties to some anesthetics that diffuse freely through the cell membrane (Hermann *et al.*, 2006). Regardless of the elusive existence of transporters for endocannabinoids, some studies have provided preclinical pharmacotherapeutic evidence that targeting the anandamide transport system using putative anandamide transport inhibitors like AM 404, may be an approach in reducing alcohol intake and in some emotional disturbances like anxiety and depression (Bortolato *et al.*, 2006; Cippitelli *et al.*, 2007). On the other hand, some studies have provided evidence

against the presence of an anandamide transporter (Glaser *et al.*, 2003). It will be premature to dismiss the existence of other endocannabinoid transporters, including 2-AG transporter, but of course the molecular identification of any endocannabinoid transporter will validate them as pharmacotherapeutic targets. Thus the mechanisms of action of endocannabinoid transporters, if they exist remain to be elucidated and whether or not 2-AG transporters and other endocannabinoid transporters exist.

VII. Variations in Cannabinoid Receptor Genes: Functional and Pharmacotherapeutic Implications

There is accumulating physiological evidence for the existence of other subtypes CBRs different from the currently known CB1 and CB2 receptors. The vanilloid receptor 1 (VRI), the site at which capsaicin in hot chili peppers acts, is a site that anandamide is a full agonist. As anandamide is a partial agonist at the CBRs, some have suggested that vanilloid (VR1) receptor be classified as a CBR subtype—may be CB3. In fact the endocannabinoid that is a full agonist at the CBRs is 2-arachidonyl glycerol (2-AG) (Gonsiorek *et al.*, 2000; Sugiura *et al.*, 1999, 2006). The GPR55 has been suggested as a CBR that increases intracellular calcium and inhibits mM current (Lauckner *et al.*, 2008). However, using a strategy for defining CBR functional fingerprints from mutagenesis and molecular recognition literature data, it was noted that hGPR55 does not appear to share similar fingerprint with the hCB1R and hCB2R (Petitet *et al.*, 2006). While this could not be considered as a proof to exclude GPR55 from the CBR family, the data from other studies strongly suggests that GPR55 is a specific functional receptor for lysophosphatidylinositol receptor (Henstridge *et al.*, 2008; Oka *et al.*, 2007). It has also been reported that 2-arachidonoyl-sn-glycero-3-phosphoinositol, a molecular species of lysophosphatidylinositol, is a possible natural ligand for GPR55 (Oka *et al.*, 2008). Thus far, it appears that GPR55 is quite distinct from other GPCRs and represents an intriguing and unique therapeutic target whose functional receptor requires further validation and characterization (Henstridge *et al.*, 2008).

We have reported that the human CB1R have a number of splice variants, which may in part account for the myriad behavioral effects of smoking marijuana (Fig. 1 and Table II). Some effects may include actions at CB2-Rs that have received much less attention than CB1-Rs. However, we and others have now identified and characterized glial endothelial and neuronal CB2-Rs in the brain. Nonetheless, many features of the CB2R *CNR2* gene structure, regulation and variation remain poorly characterized compared to the CB1-Rs. In humans the *CNR2* gene is reported to consist of a single translated exon flanked by 5′ and 3′

untranslated regions and a single untranslated exon (Sipe *et al.*, 2005) (Fig. 2). Most regions of the *CNR2* gene are highly conserved, but the human has glutamine at position 63 (Karsak *et al.*, 2005, 2007; Sipe *et al.*, 2005) and another SNP H316Y has been reported and linked to autoimmune disorders (Karsak *et al.*, 2007; Sipe *et al.*, 2005). There has been little or no data on the role of CB2-Rs in neuropsychiatric disorders. However, in neurological disorders associated with inflammation, the expression of CB2-Rs has been reported in limited populations of microglial including plaque-associated glia in Alzheimer's disease brains (Nunez *et al.*, 2004; Pazos *et al.*, 2004). Indeed our studies provide the first evidence for a role of CB2-Rs in depression and substance abuse (Ishiguro *et al.*, 2007; Onaivi, 2006; Onaivi *et al.*, 2006a, 2008a,b). We and others have identified splice variants of the human CB1-Rs and CB2-Rs but have thus far been poorly characterized for functional specificity apart from the broad roles associated with CB1-R and CB2-R subtypes. Alternative splicing of RNAs appears to be more common than previously thought in people, and can generate a variety of proteins, with most genes producing at least two variants. The characterization of CBR variants will add validity to the functional evidence for the existence of multiple CBR subtypes. It has been demonstrated *in vitro* that amino-terminal processing of the hCB1-R may involve rapid N-terminal truncation in the cytoplasma prior to translocation to the endoplasmic reticulum membrane. It was suggested that such a truncation process might be a way to create a novel type of CB1-R isoforms but exactly how the truncated CB1-R may be formed and how the processing is regulated remains to be determined (Nordstrom and Andersson, 2006), In comparison to the monoaminergic system, the application of modern techniques to cannabinoid research is new. For example molecular cloning has revealed the presence of serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes, which can be subdivided in seven subfamilies (Gerhardt and van Heerikhuizen, 1997), and 15 serotonin (5-HT) receptor subtypes and growing. Like 5-HT receptors that include ligand-gated ion channels, it has been suggested that the VR1 receptor should be reclassified as a CBR subtype since the endocannabinoid, anandamide is a full agonist at VR1 receptor, but acts as a partial agonist at CB1-s. New knowledge on cannabinoid transcriptional and posttranslational modifications, such as alternate splicing and perhaps RNA editing may indicate formation of multiple proteins could unravel specific mechanisms associated with numerous behavioral and physiological effects of marijuana use. The cloning and sequencing of CB1-R *CNR1* gene from 62 species has also been reported by Murphy *et al.* (2001) and awaits full characterization. As predicted here the identification and characterization of these putative CBR isozymes and different elements of the endocannabinoid system may reveal novel targets for medication development. However, the limitless signaling capabilities and the endless complexity of the cannabinoid system require continuous intensive investigation.

VIII. Genetic Basis of Cannabis use and Dependence

The question of physical and psychological addiction to marijuana is no longer a debate as the significant progress in marijuana-cannabinoid research have applied modern techniques using new molecular probes that were previously unavailable to resolve the issue of addiction to marijuana. There is substantial evidence in favor of heritable factors influencing the liability to cannabis use behaviors (Agrawal *et al.*, 2008). Furthermore, previous twin studies indicate that there is a role of genetic influences on early stages of cannabis use, such as lifetime history of use, early onset use and frequency of use, but no conclusive replicable genomic locations explains these phenotypes for cannabis use behaviors in all populations (Agrawal *et al.*, 2009). And of course environmental factors and the availability of cannabis is undoubtedly a contributing factor. Other studies also suggest the presence of reliable and empirically valid withdrawal syndrome for marijuana use and that genetic factors and heritability to cannabis use account for a significant portion of variance in use, abuse, and dependence (Haughey *et al.*, 2008). Thus, it has been demonstrated that marijuana withdrawal after abstinence and cue-elicited craving in humans are associated with polymorphisms in *CNR1* and fatty acid hydrolase (*FAAH*) genes (Haughey *et al.*, 2008). In humans the above study indicates that abstinence from marijuana use precipitates withdrawal in daily smokers, making craving and withdrawal variables as possible cannabis dependence endophenotypes (Haughey *et al.*, 2008). Marijuana dependency syndrome is similar to, than different from the more recognized substance dependence syndromes (Budney, 2006). In addition, animal models allowing the evaluation of genetic and neural correlates of marijuana-cannabinoid dependence and abuse potential have now been developed. While the question of marijuana-cannabinoid dependence had been controversial, the discovery of rimonabant, the CB1-R antagonist, precipitated withdrawal in rodent models indicative of cannabinoid dependence (Cook *et al.*, 1998). Similar questions are no longer posed about cigarette and opiate dependence. As this review focuses on the molecular and genetic basis of the biological effects of marijuana and the cannabinoid system, we cannot dismiss the larger question and current debate about the consequences of medicinal and recreational use of marijuana. Thus, is there evidence for the existence of a diagnosable cannabis specific withdrawal syndrome in human users? This issue has been addressed by the review of the published literature into cannabis withdrawal symptoms in human users (Smith, 2002). Long-term marijuana use can lead to addiction for some people; that is, they use the drug compulsively even though it often interferes with family, school, work, and recreational activities. Along with craving, withdrawal symptoms can make it hard for long-term marijuana smokers to stop using the drug. People trying to quit report irritability, difficulty sleeping, and anxiety.

They also display increased aggression on psychological tests, peaking approximately 1 week after they last used the drug. Tolerance, which varies with the effects of marijuana and cannabinoids, has been attributable to pharmacodynamic changes that may be associated with CBR downregulation and/or receptor desensitization (Grotenhermen, 2004). Although withdrawal symptoms from chronic dosing with high doses of THC in humans are usually mild, the risk for physical and psychic dependence is low compared to opiates, tobacco, alcohol, and benzodiazepines (Grotenhermen, 2004). Converging evidence indicate that gene–environment interaction are contributing factors to marijuana problem use in adolescence (Agrawal and Lynskey, 2008; Young *et al.*, 2006) as gender and early onset of use is a well-established factor for the progression from use to abuse and dependence (Bucholz *et al.*, 2000). After the discovery of endocannabinoid system in the brain, it is now known that it plays a crucial role in regulating the effects of addictive substances, including psychostimulants like nicotine, cocaine, opioids, alcohol, and benzodiazepines. While it is obvious that the initial targets of abused drugs are different, evidence indicates a converging common liability of the endocannabinoid system underlying the dependence to abused drugs. Thus, new findings and hypothesis are investigating the ECS as a common neurobiological mechanism underlying drug addiction (Onaivi, 2008). A number of studies have now shown that CBR genes are not only associated with polysubstance abuse but with other individual drug dependences and with psychiatric disorders (Chen *et al.*, 2008). Therefore, the powerful neuromodulatory action of the ECS on synaptic transmission and interactions with the effects of addictive substances may be an important natural regulatory mechanism for drug reward and therefore a target for the treatment of addictive disorders.

In considering the genetic basis of marijuana use and dependence, it is important to take into account that cannabis and cannabinoid medicinal preparations may no longer be exclusively considered recreational drugs, as they have a number of therapeutic applications and thus it has become of enormous interest to examine their mechanism(s) of action. Data from family, twin, adoption and multivariate genetic studies have provided evidence that cannabis use, abuse, and dependence can be attributed to polygenetic and environmental factors (Agrawal and Lynskey, 2006). We now know that marijuana or cannabis use has dependence liability because of the physical and psychological effects that leads to the classical relapse cycle of abused substances. Our new understanding from linkage and association studies continues to provide substantial evidence for the heritability of cannabis use, abuse, and dependence. There is therefore a role of the endocannabinoid system in the sequelae of environment–genetic interaction for marijuana use and dependence. Emerging evidence comes from accumulating knowledge on cannabinoid genetics; animal modeling of key features of marijuana-cannabinoid dependence that can be evaluated in electrophysiological, pharmacological and linkage and association, and immunohistochemical studies.

For example, behavioral cannabinoid withdrawal syndrome and gene transcriptions along with hormonal alterations have been described in the mouse model following cessation of treatment from CP-55,940, a cannabinoid agonist (Oliva *et al.*, 2003). It is important to note that from mice to human subjects the gene alterations are not limited to *CNR* genes only. The polygenic factors have been defined to mean that many genes with varying effect sizes are responsible for the genetic variation associated with marijuana use and dependency (Agrawal and Lynskey, 2006). Thus, there is accumulating genomic data from the identification of candidate genes and variants, and changes in gene transcription associated with the genetic basis of cannabinoid actions. Such genetic predisposition may include genes that regulate drug sensitivity and predisposition, influence sensation-seeking, or influence general problem behavior (Agrawal and Lynskey, 2006). It appears that a pharmacogenetic approach in drug addiction has potential for improving treatment outcome as gene variants that affect pharmacodynamic and pharmacokinetic factors are identified. It appears gene mutations might guide pharmacotherapeutic agent of choice for treatment of drug addiction (Haile *et al.*, 2008).

While, the etiology of marijuana dependence is a complex interaction of psychological, environmental, and biological factors associated with genetic vulnerability, the study of the contribution of genetic and environmental factors in cannabis dependence is further complicated by polysubstance use. Therefore overlapping gene–environment interactions may contribute to comorbid use, abuse, and dependence on marijuana and other drugs. Comorbidity and genetic loading studies indicate that adults using marijuana on a daily basis have a high frequency of co-abuse of other substances, like nicotine and alcohol. Therefore, an individual who becomes dependent on marijuana will exhibit tolerance, withdrawal reactions, craving and relapse, preventing cessation of use that characterizes the addiction cycle. Comorbidity with other psychiatric disturbances such as depression, panic attacks, anxiety, and antisocial personality disorders (Comings, 1996) have also been reported. Many studies have therefore examined the genetic and environmental contributions to risk of cannabis dependence. The notion of combined use of multiple substances with dependence potential sharing a common genetic influence has been an enduring issue in addiction research (Crabbe, 2002). However, each abused substance appears to be linked to independent genetic influences that have made it difficult to identify addiction specific gene(s), or if an addictive personality gene(s) exists. The questions exist about marijuana dependence and what genes are involved in the continuous use, withdrawal syndrome, and relapse to cessation of use? Our current knowledge shows that specific CBRs, which are encoded by CBR *CNR* genes, are activated by smoking marijuana or by the administration of cannabinoids. The myriad behavioral, physiological, and modulation of programs of gene expression following activation/inhibition of the CBRs is the subject of intense investigation.

While there is increasing evidence that genetic factors are involved in marijuana dependence through studies of familial transmission of marijuana use, abuse, and dependence (e.g., Hopfer *et al.*, 2003), new gene–environment interplay, and linkage association studies, genome scans, and molecular genetic analysis are providing insights into novel phenotypes and endophenotypes of marijuana use, dependency, and comorbidity. Therefore, risk for cannabinoid dependence is likely to be the result of a number of genes, each contributing a small fraction of the overall risk, with allelic variants contributing to different aspects of marijuana dependence. One commonly used approach to identify genetic influences on addictions is the candidate gene approach, which directly tests the effects of genetic variants of a potentially contributing gene in an association study (Kwon and Goate, 2000). The age of onset of marijuana dependence and smoking may also be influenced by genetic risk factors, because it may be unlikely for a 70-year-old man or woman who never initiated smoking marijuana to become dependent on marijuana. As with tobacco and alcohol dependence, application of oligogenic linkage analysis and determination of endophenotypes or trait markers (Tyndale, 2003), may improve the ability to identify specific genetic variants contributing to marijuana dependence. Perhaps as with genetic association studies of alcoholism, similar problems may be encountered with the candidate gene approach in marijuana dependence because of the complexity of addictions (Buckland, 2001).

We have demonstrated that alternative *CNR1* transcripts exist in the CNS that is associated with poly substance abuse in humans (Zhang *et al.*, 2004). Other studies have reported a combined drug dependence phenotype to regions on chromosome 3, 9, 11, and 20. It appears that the genome-wide scan for loci influencing cannabis dependence symptoms on chromosome 3q21 suggest that the region may contain a quantitative trait loci linking cannabis dependence and other substance use disorders (Hopfer *et al.*, 2007). Chromosomes 1 and 6 that habors the CB-R genes can be assumed from our data are genomic regions of considerable interest not only in polysubstance abuse but as possible targets for future mapping in depression and other neuropsychiatric disorders. The CB1-R *CNR1* gene, which is a target of marijuana smoke, is amongst the addiction "hot spots" in the human chromosomes. It is also a logical candidate gene for vulnerability toward developing symptoms of cannabis dependence. The central role of the cannabinoid system in the effects of other abused substances as demonstrated by CB1 receptor mutant mice and from association studies make CB1-R locus, a strong candidate with variants that might contribute to individual differences in drug abuse vulnerability (Zhang *et al.*, 2004). The retrograde action of endocannabinoids on the inhibition of classical neurotransmitter release (e.g., on glutamate, GABA, serotonin, dopamine neurotransmitters) may be differentially modified by genetic variants of the CB1-Rs, which are the main molecular targets for the endocannabinoids and marijuana-cannabinoids. Until recently, many features of the CB1 *CNR1* gene's structure, expression, regulatory regions,

polymorphisms, haplotypes, and association with polysubstance abuse were poorly defined. Some studies have examined CB1-R *CNR* genomic variation and association studies have used known markers and increasingly CB1-R associated SNPs have appeared (NCBI:http://www.ncbi.nlm.nih.gov). For example two such transcripts variants derived from the 3′ end, encoding a longer isoform and short isoform, missing a segment near the 5′ end results in a frameshift with different N-terminus compared to the long isoform was reported. Our recent studies have now demonstrated the existence of novel exons, splice variants and candidate promoter region sequences that confer reporter gene expression in cells that express CB1-Rs (Zhang *et al.*, 2004). The results from the association studies show common human CB1 polymorphisms that reveal patterns of linkage disequilibrium in Caucasian and African–American individuals; while a 5′ CB1/*CNR1* "TAG" haplotype displays significant allelic frequency differences between substance abusers and controls in Caucasian, African–American, and Japanese samples. A common CBR haplotype associated with fewer cannabis dependence symptoms in adolescents who have experimented with cannabis has also been reported (Hopfer *et al.*, 2006). This new work of improved definition of the CB1/CNR1 locus and its variants therefore adds to the interesting features of regulating the neural circuits important for the reinforcing effects of most abused substances with a critical role in addiction vulnerability (Zhang *et al.*, 2004). The involvement of CB1-R variants in other phenotypes may have significant roles in cannabinoid pleiotropy as the effects of activation of the CB1-R have been implicated in a number of behavioral functions. The emerging structure of the genomic CB1-R *CNR1* gene is complex with multiple exonic sequences, splice variations, polymorphisms, and regulatory regions that is the central feature of the endocannabinoid physiological control system. Certainly, understanding the molecular structure and regulation of genes involved with the endocannabinoid system might be a first step in characterizing the genetic basis of smoking marijuana. Unfortunately, our knowledge of how genes affect complex traits is currently poorly understood.

IX. Functional Implication of Cannabinoid Pharmacotherapeutics

The use of cannabis for both recreational and medicinal purposes dates back for thousands of years. In recent times there has been increased attention that marijuana should be legalized for medicinal use in AIDS, cancer, obesity, multiple sclerosis, and other medical conditions where patients might benefit from the pharmacological effects of cannabis. Synthetic cannabinoids such as dronabinol, marinol, and nabilone already have an established use as antiemetics in nausea and vomiting associated with cancer chemotherapy. The reported beneficial

effects in cancer and AIDS patients might reflect improved weight gain, owing to the well-documented antiemetic and appetite stimulating effects of cannabinoids. This might be a major advantage for cancer patients undergoing rigorous chemotherapy, or advanced AIDS patients. The potential draw back in the use of cannabis for medicine has been the psychoactivity induced by smoking marijuana and the potential for abuse and dependence. As presented in this review, there have been major advances in understanding the genetic and thus the biological mechanisms whereby cannabis and cannabinoids interacts with the brain in producing psychoactive and potentially pharmacotherapeutic effects (Onaivi *et al.*, 2006b). The discovery of specific genes encoding CBRs activated by smoking marijuana, and the finding of endocannabinoids, which also the receptors, have transformed cannabinoid research into main science with significant implications for human health and disease. Thus the various components and elements of the endocannabinoid system, including cannabinoid genes, receptors, their endogenous ligands and metabolizing, and synthetic enzymes are potential pharmacogenetic and pharmacotherapeutic targets. We have exhaustively documented our current knowledge and functioning of the naturally occurring marijuana—like substances in human biology (Onaivi *et al.*, 2002b, 2006b). We now know that the existence of the endocannabinoid system, directly impacts human development, health, and therefore is a target in disease conditions. The ubiquitous distribution of this system throughout the entire human physiology makes the components of the endocannabinoid system a target for therapeutic intervention during cell development, cancer, immune disorders, in reproduction and digestion, CNS function and dysfunction, as in mental illness, and neurodegenerative diseases (Onaivi *et al.*, 2002a, 2006b). In addition to CBR—independent action of antitumor effects, evidence now exists that phytocannabinoids and endocannabinoids inhibit the proliferation of some tumors and cancer cells by mechanisms involving inhibition of angiogenesis and the activity of K-ras oncogene products. It has been shown that cannabinoids and endocannabinoids have some role in cancer cell growth and could be potential adjuncts as targets in the treatment of cancer.

Interestingly, although cannabis is widely used as a recreational drug in humans, only a few studies have revealed an appetite stimulant potential of cannabinoids in animals. However, evidence for the aversive effects of Δ^9-THC, WIN 55,212–2, and CP 55,940 on appetite is more readily obtained in a variety of tests. The blockade of CB1-R by rimonabant impaired the perception of the appetitive value of positive reinforcers (cocaine, morphine, and food) and reduced the motivation for sucrose, beer and alcohol consumption, indicating that positive incentive and/or motivational processes could be under a permissive control of CBR-related mechanisms. With the implication of the ECS in appetite regulation and critical roles in reward pathways, it appears that

blockade of the CB1-Rs could suppress the powerful cravings that drive the cycle of addiction. Taking together, with the appearance of the endocannabinoid system in development, its presence in breast milk, and its activation by drug and alcohol use, including smoking marijuana, may provide a target to control cravings for food and chemical dependency. Rimonabant (Accomplia) was approved in Europe in 2006 for use in obesity and other metabolic indications but was suspended in 2008 because of suicides and depression as side effects in vulnerable individuals. It must be noted that in many obese patients rimonanbant was most effective as an antiobesity medication and the other complications including diabetes. The abundance of ECS in the human body and brain in comparison to other G-protein-coupled receptors support the critical role of this system in most physiological and biochemical functions that affects behavioral changes after smoking marijuana. Furthermore, there are misconceptions that people addicted to drugs lacked willpower and were morally weak, or that abused drugs release dopamine in the brain's reward centers to produce pleasure and euphoria leading to addiction in vulnerable individuals (Salamone *et al.*, 2005; Spanagel and Weiss, 1999). We now know that drug addiction is a brain disease and experiments have been conducted to test the endocannabinoid hypothesis of drug reward and addiction and whether or not CBRs ligands might be useful as drug abuse pharmacotherapeutic targets (Onaivi, 2008). Our data have established a frame work for an endocannabinoid hypothesis of drug reward and addiction and as a target for the development of cannabinoid therapeutic agents for substance abuse disorders. Thus, several cannabinoid-mediated effects are of interest for therapeutic applications. However, whether or not the antagonism of the CBRs will be effective in treating individuals with problematic and uncontrollable marijuana smoking addiction and who wishes to quit remains to be demonstrated. Furthermore, endocannabinoids and other endogenous fatty acid ethanolamides with their roles in sleep and inflammation are emerging as a new important biological signaling molecules and targets for therapeutic intervention. Other targets for potential development of therapeutic agents are cannabinoid uptake inhibitors, if they exist. When the physiological role of CBRs and endocannabinoids are established, it is likely that other therapeutic targets may be uncovered. Based on what we currently know about the anatomical distribution of CBRs and endocannabinoids and from marijuana users, disorders associated with memory and motor coordination may benefit from novel cannabinoids. Similarly, CBRs and endocannabinoids can be targeted in immune disorders and blood pressure regulation. This is to be expected as many novel drugs are based on chemical modifications of transmitters. Therefore, it seems reasonable to expect that, as with other receptor transmitter systems, excess or lack of CBRs or endogenous ligands may be the cause for disorders in the CNS or those associated with the immune and other peripheral organs.

X. Conclusions and Future Directions

The recent significant progress on marijuana cannabinoid research has lead to new understanding about the biological effects and the unique therapeutic possibilities of targeting the endocannabinoid system which are activated by cannabinoids, endocannabinoids, and marijuana use. In just a few years since the discovery of endocannabinoids that serve as cannabinoids *in vivo*, these lipid signaling molecules and endolipids have been shown to participate in a broad array of physiological and pathological processes. However, the impact of "lipidomics" and these new lipid signaling pathways in modulating behavioral neurobiology and aspects of drug addiction is of current interest. It is now clear that marijuana use is addicting in vulnerable population. Just like most medications that are not benign, benzodiazepines, opiates, and marijuana have side effects that accompany their therapeutic use. Unlike, opiates the intensity of the craving and withdrawal reactions appears to be mild and vary from individual to individual with marijuana dependence. Thus we can look forward to a bright future of discoveries concerning the role of endocannabinoids in brain function, immune function, reproductive function, and emotional behavior, as well as the discovery of potential medicines to improve the health of many who suffer from various disorders. Marijuana (cannabinoid) research appears to have a solid scientific background for significant contribution in understanding human biology and in the development of cannabinoid based therapeutics. The only medical uses for which there has been rigorous scientific evidence are in the treatment of sickness associated with cancer chemotherapy and to counter the loss of appetite and wasting syndrome in AIDS (Iversen, 2000). There is however scientific evidence for the potential therapeutic use of cannabinoids for the treatment of psychomotor disorders, including spasticity, multiple sclerosis, epilepsy, disk prolapse, ADHDs, and pain is no longer a matter of speculation. It is a matter of time when new medications for the pharmacological manipulation of the link between endocannabinoid and other neurotransmitter systems can be developed as targets for the numerous indications of marijuana and endocannabinoid ligands. In the absence of clinical data on the abuse liability of endocannabinoids, the preclinical data indicate that like marijuana, the endocannabinoids may have minimal abuse liability in humans. The safety profile of THC, the active ingredient of cannabis, is good. It has low toxicity in both the short and long term. As concluded by Iversen (2000), marijuana is not a completely benign substance. It is a powerful drug with a variety of effects. However, except for the harms associated with smoking, the adverse effects of marijuana use are within the range of the side effects tolerated for other medications. Unraveling the genetics of the endocannabinoid system that is a target for smoking marijuana is dependent on further understanding of the complex *CNR1*, *CNR2*, and *CNRn* gene structure and their associated

regulatory elements that are still poorly understood. It is concluded, that with cannabis use coded in our genes, research on the use of cannabis as medicine with a clear focus on scientific outcome as discussed in this review, rather than on paranoia or repression of cannabis use is the way forward. The perceived dangers of cannabis use no longer outweigh its potential beneficial effects to certain groups of chronically ill patients.

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