CNS Effects of CB2 Cannabinoid Receptors

Emmanuel S. Onaivi[*,1,2], Hiroki Ishiguro[2,3], Qing-Rong Liu[2], Jian-Ping Gong[2], Patricia Tagliaferro[4], Alicia Brusco[4], Tadao Arinami[3] and George R. Uhl[2]

[1]*William Paterson University, Wayne, USA*

[2]*Molecular Neurobiology Branch, National Institute on Drug Abuse, NIH, Baltimore, USA*

[3]*Department of Medical Genetics, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, Japan*

[4]*Instituto de Biologia Celular y Neurociencias Prof. "E. De Robertis", Facultad de Medicina, Universidad de Buenos Aires, Argentina*

Abstract: Cannabinoids, endocannabinoids and marijuana use activates two well characterized cannabinoid receptors (CBRs), CB1-Rs and CB2-Rs. The expression of CB1-Rs in the brain and periphery has been well studied, but CB2-Rs have received much less attention than CB1-Rs. CB2-Rs were previously thought to be predominantly expressed in immune cells in the periphery and were traditionally referred to as peripheral CB-Rs. We and others have now demonstrated the presence of CB2-Rs in neuronal, glial and endothelial cells in the brain, and this warrants a re-evaluation of the central nervous system (CNS) effects of CB2-Rs. However, many features of CB2-R gene structure, variants and regulation remain poorly characterized compared to the CB1-R. To further improve understanding of the role of CB2-Rs in the brain, we hypothesized that genetic variants of CB2-R (*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. In this review we show that our data and those of others reveal that CB2-Rs are expressed in neurons in the brain and play a role in depression and substance abuse beyond neuro-immuno-cannabinoid activity.

Keywords: *CNR2*, CB2 cannabinoid receptors, neuronal and glial distribution, brain.

INTRODUCTION

Cannabinoids are the constituents in marijuana and endocannabinoids (eCBs) are the endogenous marijuana-like substances found in animals and humans [1]. Recent advances in cannabinoid research indicate the existence of an endocannabinoid system (ECS) consisting of genes encoding cannabinoid receptors (CB1-Rs and CB2-Rs), their endogenous ligands eCBs and proteins that synthesize and degrade these eCBs. Both CB1-Rs and CB2-Rs are distributed in the brain and peripheral tissues and are activated by endocannabinoids, and cannabinoids, the active constituents in marijuana [1]. For many years it was thought that marijuana use, phytocannabinoids and eCBs act by activating brain-type cannabinoid receptors (CBRs) called CB1-Rs, and that a second type called CB2-Rs were found in peripheral tissues and mainly in immune cells and were referred to as peripheral CB2-Rs. This was because many investigators were not able to detect the presence of neuronal CB2-Rs in healthy brains [2-4], but CB2-R expressions were demonstrated in rat microglia cells and other brain-associated cells during inflammation [5-10]. Despite the evidence that CB2-Rs might be present in the CNS, the expression of neuronal CB2-Rs in the CNS has been much less well established and characterized in comparison to the expression of abundant brain CB1-Rs. We and others and many recent studies have reported the discovery and functional characterization of neuronal brain CB2-Rs (Table **1**).

EVIDENCE FOR THE FUNCTIONAL PRESENCE OF CB2 CANNABINOID RECEPTORS IN BRAIN

A number of studies from mice to human subjects, using a variety of techniques including those used in pain models, histological, immunohistochemical, electron microscopy, molecular biological, behavioral and pharmacological, pharmacological MRI, cerebral occlusion and hemicerebellectomy, transgenic and cell culture studies show the functional presence of CB2-Rs in neural progenitor cells, neurons, glial and endothelial cells [11-14]. In this review we address the CNS effects of CB2-Rs and its possible involvement in drug addiction and neuropsychiatric disorders. While the role of CB2-Rs in CNS disturbances involving neuroinflammation and neuropathic pain has been extensively reported [15], our studies provided the first evidence for a role of CB2-Rs in depression and in the effects of substance abuse [15-17]. The controversy of the functional expression of brain neuronal CB2-Rs remains because *CNR2* gene and CB2-Rs have received much less attention than CB1-R (*CNR1*) gene and CB1-Rs. Although the expression of CB1-Rs in the brain and periphery has been well studied, many features of *CNR2*

*Address correspondence to this author at the Department of Biology, William Paterson University, Wayne, NJ 07470, USA; Tel: +1 973-720-3453; Fax: +1 973-720-2338; E-mail: onaivie@wpunj.edu

1876-5238/09

Table 1. Immunohistochemical Distribution of CB2 Receptors in the Nervous System

Areas of the Nervous System	Cellular Type	Subcellular Localization	References
Olfactory bulb and tubercle	Neurons		[25]
Cerebral cortex: orbital, visual, motor and auditory cortex	Pyramidal neurons layers III and V		[25]
Hippocampus: (CA2 and CA3 areas) Strata oriens and radiatum	 Pyramidal neurons and interneurons Neuropil	 Neuronal cytoplasm and dendrites	 [12, 25] [27]
Corpus callosum	Glial cells		[25]
Midbrain areas: Periaqueductal gray matter, paralemniscal, paratrochlear and red nuclei. Substantia Nigra (pars reticulata)	 Neurons Neurons	 Dendrites, axons and axon terminals	[25, 35]
Cerebellum: Molecular layer Purkinje cells layer Granular layer	 Parallel varicose fibers and neuropil Neurons Mossy fibers and neuropil		 [26, 37] [25, 37, 38] [26, 37]
Brainstem nuclei: Vestibular and cochlear Nuclei. Parvocellular reticular nucleus, spinal trigeminal tract nucleus Dorsal motor nucleus of the vagus, nucleus ambiguous and spinal trigeminal nucleus	 Neurons	 	 [25, 37] [25] [24]
Pineal gland	Pinealocytes and intrapineal nerve fibers		[28]

gene structure, regulation and variation remain poorly characterized in comparison to the *CNR1* gene encoding the CB1-Rs. This poor characterization of *CNR2* gene structure and variants hampers progress in the determination of the functional role of CB2-Rs in a number of CNS disturbances. Additionally, the CNS presence of CB2-Rs may no longer be a debate, but the neurobiological basis for CB2-R physiological activity and its interaction with or without CB1-Rs remain to be determined. Another complication is the pharmacological MRI (phMRI) study in rats which did not observe changes in brain activity when AM1241, a CB2-R selective agonist was administered [13]. It concluded that if CB2-Rs are present in the naïve brain, they are not functionally detectable using phMRI methodology. However, they pointed out that the phMRI data in naïve rats do not differentiate between the potential lack of CB2-R expression and the presence of non-functional CB2-Rs in regions of the brain [13]. However, an overwhelming number of studies now document CB2-R expression in neuronal, endothelial and glial cells (Table **1**). For example studies in human tissue samples from Alzheimer's disease subjects revealed that CB2-Rs are selectively over expressed in the microglial cells that are associated with Aβ-enriched neuritic plaques [18]. Data obtained *in vitro* and from animal models demonstrated the inducible nature of CB2-Rs under neuroinflammatory conditions and suggests that the upregulation of CB2-Rs is a common pattern of response against different types of chronic human brain neuropathology [19]. Mounting evidence also shows that CB2-Rs and its gene variants may play possible roles in neuroinflammation occurring in multiple sclerosis, traumatic brain injury, HIV-induced encephalitis, Alzheimer's, Parkinson's and Huntington's diseases [19]. A central neuronal but glial independent neuroprotection by CB2-R activation was reported to counteract apoptotic cell death that is induced by remote axonal damage that is achieved through PI3K/Akt signaling [14]. Functional interactions between forebrain CB2-R and mu-opioid receptor (MOR) were demonstrated [20] and CB2-R antagonist SR144528 was reported to decrease MOR expression and activation in mouse brain stem [21]. Upon our discovery of the presence and functional expression of cannabinoid CB2-Rs in the brain [22], most recent studies have confirmed that CB2-Rs are present in both cultured neural cells and the nervous system of several mammals such as rodents, monkeys and humans under normal conditions [23]. Thus CB2-Rs have been implicated in the control of fundamental neural cell processes, such as proliferation and survival. It was therefore suggested that manipulating CB2-Rs might be useful for delaying the progression of neurodegenerative disorders and inhibiting the growth of glial tumors [23]. CB2-Rs have also been shown sub serving differential physiological roles in other neuroanatomical sites such as the brain stem, cortex, cerebellum, PAG, substantia nigra, hippocampus, thalamus, pineal gland and pinealocytes [7, 8, 24-27]. CB2-Rs in the pineal gland along with other components of the ECS may be involved in the control of pineal physiology [28]. Gender-dependent changes on the expression of hippocampal CB1-Rs and CB2-Rs were demonstrated in the early maternal deprivation model in neonatal rats [27]. While CB1-Rs remain one of the most ubiquitous G-protein coupled receptors in the mammalian brain, we have described the multifocal distribution of CB2-Rs albeit at lower levels than the CB1-Rs in neuronal and glial processes in a number of brain areas [25]. These multifocal distributions and the presence of CNS brain CB2-Rs

suggest a need to re-evaluate the role of these receptors in neurotransmission. It is important to understand the role of CB2-Rs and its gene variants in the CNS and its possible involvement in drug addiction and neuropsychiatric disorders. Research, however, on the involvement of CB2-Rs in neuroinflammatory conditions and in neuropathic pain has advanced more than other areas in neuropsychiatry and drug addiction. Therefore, improved information about *CNR2* gene and its human variants might add to our understanding, not only of the role of CB2-Rs during neuroinflammatory conditions in the CNS but also beyond neuro-immuno-cannabinoid activity.

CNR2 GENE STRUCTURE AND CB2-RECEPTOR SUB-TYPE SPECIFICITY

The *CNR2* cannabinoid gene structure has been poorly defined, thus far. However, many features of the cannabinoid *CNR2* gene structure, regulation and variation are beginning to emerge with the discovery and functional identification of CB2-Rs in mammalian CNS. This prior poor definition could be related to the previously held view that *CNR2* gene and CB2-Rs were not expressed in the brain but mainly in immune cells. It was therefore less investigated for CNS roles except for the association with brain cells of macrophage lineage. This has begun to change [15]. The human *CNR2* gene and its mouse and rat orthologs are located on chromosomes 1p36, 4QD3 (Figs. **1**, **2**) and 5Q36, respectively. Genome-sequencing projects have also identified *CNR2* genes in chimpanzee, dog, cow chicken amphibian, puffer fish, and zebra fish. It appears that the human, rat, mouse and zebra fish genomes contain two isoforms of CB2-Rs that have differential distribution patterns in the brain and peripheral tissues. Interestingly the puffer fish *Fugu* rubripes has two *Cnr1* genes and one *Cnr2* gene in contrast to zebra fish *Danio* rerio that has two *Cnr2* genes and one *Cnr1* gene.

The complete gene structure, 5'- and 3' –UTR, and transcription initiation sites of human CB2-Rs have not been fully characterized [22, 29], until now. After we and others identified and reported mouse CB2-R expression in brain regions [24 and 25], the specific expression of human or mouse CB2-R isoforms in brain regions was not known. But the published evidence showed significant species differences of CB2-Rs in human, mouse and rat in terms of peptides, mRNA sizes, gene structure and pharmacology [2, 30, 31]. Therefore, the discrepancies on the CB2-R mRNA sizes in the literature indicated incomplete gene structure of *CB2* gene in different species or polymorphism in the same species. We have discovered a novel human *CNR2* gene promoter encoding testis (CB2A) isoform starting exon located ca 45 kb upstream from previously identified promoter encoding the spleen isoform (CB2B) [32]. The size of the newly identified hCB2A isoform is about twice as large as the previously identified human hCB2B gene. The 5' exons of both CB2-R isoforms are untranslated 5'UTRs and alternatively spliced to the major protein coding exon of *CNR2* gene (Fig. **2**). We showed that CB2A is expressed higher in testis and brain than CB2B that is expressed higher

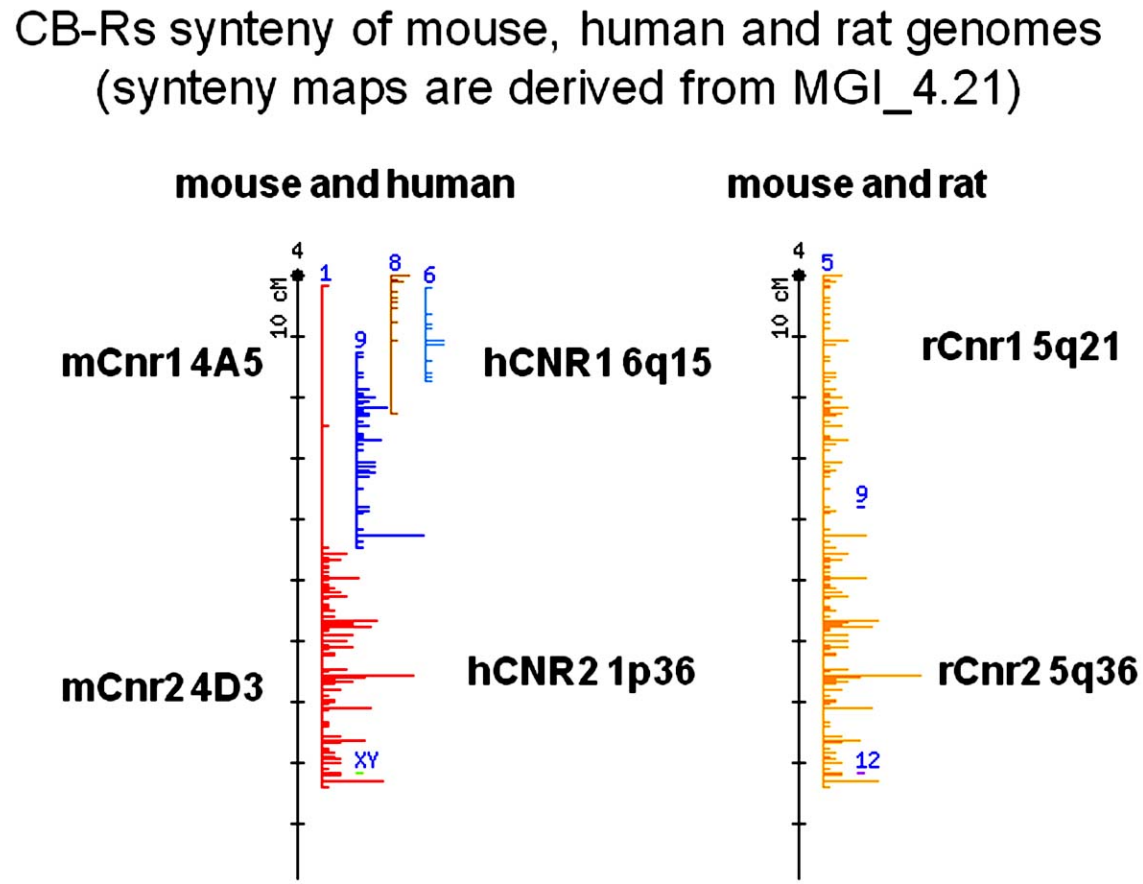

Fig. (1). Chromosomal localization of CB1-R and CB2-R *CNR* genes. The synteny map showing chromosomal locations of CB1-R and CB2-R CB genes are on mouse chromosome 4, rat chromosome 5 and human chromosome 6 and 1P36, respectively. Synteny maps are derived from MGI-4.21.

in other peripheral tissues than CB2A. Using precise probes, species comparison found that the *CNR2* gene of human, rat and mouse genomes deviated in their gene structures and isoform expression patterns and could be regulated by cannabinoid ligand treatment in the mouse model (Fig. **2**) [32]. The human *CNR2* gene is almost four times larger than the mouse and rat *Cnr2* genes. If the transcription rates are similar between human and rodents, hCB2A isoform would take much longer time to be transcribed in the testis and brain. This will be unusual because other gene orthologs between humans and mice are usually within one fold difference in genomic sizes. Our data shows that there are two forms of the CB2-Rs in human, rat and mouse with differential subtype distribution specificities in the brain and peripheral organ tissues. The promoter-specific CB2-R isoform distribution may in part explain why CB2-Rs were previously undetectable in both human and rodent brains [2, 4 and 29].

CNS CB2 RECEPTOR IMMUNOHISTOCHEMICAL DISTRIBUTION AND SUBCELLULAR LOCALIZATION

Many previous studies could not detect the expression of CB2-Rs in the brain [2, 4 and 29], because the PCR primers may not have been specific to detect CB2-R isoforms. Furthermore, the specificity of the available antibodies for both CB1 and CB2-Rs has also been controversial as some could not detect the native and in some cases the transfected cannbinoid CBR antigen, although they recognized proteins in Western blot and in immunohistochemical analysis (Fig. **3B**) [33]. There are also problems with the antibodies because of the species differences between human and rodent CB2-R genes. We have resolved some of these issues by using CB2 isoform specific TaqMan probes that could differentiate the isoform-specific expression and are more sensitive and specific than CB2 antibodies that are currently available. The controversial CB2-R brain expression could also be due to the low expression levels of CB2B isoform in brain regions and the less specific CB2-R commercial antibodies in immunohistochemical studies, especially those studies using antibodies against human hCB2 epitopes for rodent brain immunostaining. There are also problems with the use of the *Cnr2* gene knockout (ko) [34] mice in Western blots and in behavioral analysis. When we analyzed the *Cnr2* knockout mice using the three TaqMan probes against two promoters of mouse *Cnr2* gene and the deleted part of *Cnr2* gene, we found that the promoters of *Cnr2* gene ko mice were still active and that a CB2-R truncated version was expressed, indicating that the *Cnr2* gene ko mice with ablation of the C-terminal peptides of 131 amino acids [34] were an incomplete *Cnr2* gene knockout. Another mouse *Cnr2* gene ko mice that has now been generated with ablation of N-terminal peptide 156 amino acid (Deltagen, Inc. San Mateo, CA) may clarify the specificity of the antibodies that were used against the N-terminal epitopes.

Thus, contrary to prior reports that CB2-Rs were not expressed in the brain, we and others have now reported the wide distribution of CB2-Rs in brain regions (see Table **1** and Figs. **3**, **4**) suggesting a reevaluation of the role of CB2-Rs in the CNS. We and others have demonstrated that immunoblots from mouse brain and spleen lysates revealed a major band of approximately 53 kDa, with other visible bands around 37 kDa and 75 kDa (Fig. **3B**), similar to those observed by others [15, 24-28]. While the specificity of some of the available antibodies has been questioned, some of the antibodies showed very similar staining patterns in both the rat spleen and cerebellum sections (Fig. **3F-I**) [25]. CB2-R immunostaining (iCB2) was observed in many parts of the rat and mouse CNS. Most iCB2 was found in patterns consistent with nerve cells (Fig. **3C-E**). CB2-R immunoreactivity was observed in the cerebellum, hippocampus, olfactory tubercule, island of Calleja, cerebral cortex, striatum, thalamic nuclei, amygdala, substantia nigra, periaquductal gray, paratrochlear nucleus, paralemniscal nucleus, red nucleus, pontine nuclei, inferior colliculus and the parvocellular portion of the medial vestibular nucleus in the rat brain (Fig. **4**). In demonstrating neuronal presence of CB2-Rs *in vitro*, sequential double labeling was used. The hippocampal slide cultured tissue preparation was first labeled with the CB2-R antibody followed by the neuronal marker, neuron-specific enolase (NSE). The immunopositive expression was detected in perikarya and in neuronal processes as well as glial cells. In mice iCB2 was observed in the regions of cerebral cortex that were analyzed and apical dendrites of pyramidal neurons were moderately to heavily stained [15]. In addition, a moderate to dense CB2-R

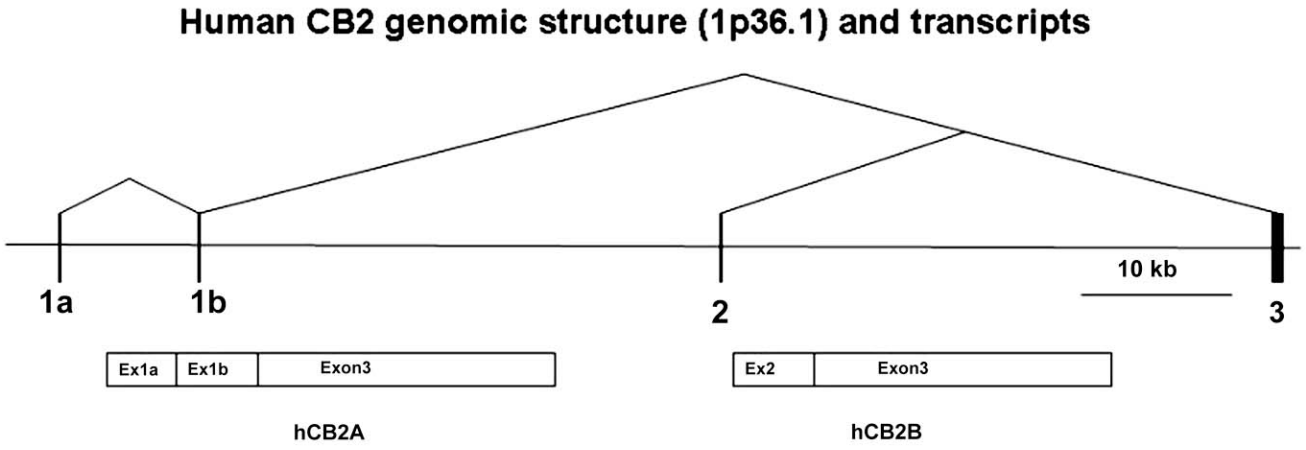

Fig. (2). The human *CB2* gene structure. Human *CNR2* gene and its mouse ortholog are located on chromosomes 1p36 and 4QD3, respectively. Most regions of the *CNR2* gene are highly conserved, but the human has glutamine and mice and rats have arginine at position 63.

immunostaining was observed in pyramidal neurons in hippocampal allocortex and some inter neurons in the stratum oriens, stratum radiatum and some glial cells also appeared to be immunostained for CB2-Rs in the mouse hippocampus. Confocal immunofluorescence image of iCB2 and NSE on neurons and the overlay of CB2-R on neurons illustrated the presence of CB2-Rs on hippocampal neurons [25].

Using mice and rats brains, the subcellular distribution of CB2-Rs in neuronal, endothelial and glial cells in the cortex, hippocampus and substantia nigra were shown using immunohistochemical electron microscopy [12, 15, 35]. In the rat brain study and in each region immunoperoxidase labeling for CB2-Rs was detected in neurons as well as in glial and endothelial cells. In neuronal cells, iCB2 was observed in somata and large and medium-sized dendrites. In the soma, iCB2 labeling was mainly associated with the rough endoplasmic reticulum and Golgi apparatus, suggesting its endogenous synthesis. In the dendrites, iCB2 labeling was observed in the cytoplasm and was associated with the plasma membrane near the area of synaptic contact with axon terminals, indicating a postsynaptic distribution of CB2-Rs. In iCB2 glial and endothelial cells, the labeling was also found to be associated with the plasma membrane. In the substantia nigra, some unmyelinated axons were immunoreactive for CB2-Rs, and CB2-R-labeled axon terminals were rarely found. In mice, electron micrographs from different cortical areas show dendrites with immunostaining for CB2-Rs. In some areas, axon terminals were not immunoreactive for CB2-Rs. The pattern of staining in most mouse cortical areas appeared to be mainly postsynaptic localization of CB2-Rs [15]. Our data therefore provided the first untrastructural evidence that CB2-Rs are mainly post-synaptic in the rat hippocampus and substantia nigra and in some cortical areas in the mouse brain [12, 15, 35]. We cannot exclude that some of the CB2-Rs may be presynaptic [26], just like CB1-Rs are not exclusively presynaptic, with some postsynaptic distribution reported [36] in the brain.

CNS EFFECTS OF CB2 RECEPTOR AND ITS POSSIBLE INVOLVEMENT IN DRUG ABUSE AND NEUROPSYCHIATRIC DISORDERS

Addiction and depression are mental health problems associated with stressful events in life with high relapse and reoccurrence even after treatment. The CNS effects of CB2-Rs had been ambiguous and controversial and its role in depression and substance abuse was unknown. We therefore have conducted studies from mice to human subjects to examine, 1). The involvement of CB2-Rs in alcohol preference in mice and alcoholism in a human population [16]. 2). The behavioral effects of CB2 cannabinoid receptor activation and its influence on food and alcohol consumption in mice [39] and 3). The involvement of brain neuronal CB2-Rs in the effects of drugs of abuse and in depression [17]. Several lines of experimental evidence support roles for the ECS in alcoholism and neuropsychiatric disorders [39-41]. We therefore tested if CB2-Rs in the CNS play a role in alcohol abuse/dependence in an animal model and then examined the association between *CNR2* gene polymorphism and alcoholism in human population. We found that mice preferring alcohol had reduced *Cnr2* gene expression in the ventral midbrain whereas the *Cnr2* gene expression was unaltered in the ventral midbrain region of mice with little or no preference for alcohol. Treatment of mice with the CB2-R agonist JWH 015, enhanced alcohol consumption in mice subjected to chronic mild stress (CMS) and the treatment with the CB2-R antagonist AM630, reduced the stress-induced increase in alcohol consumption. This CB2-R agonist or antagonist effect was absent in normal mice that were not subjected to CMS. To further understand the physiological relevance of the expression of CB2-Rs and their gene transcripts, we examined the expression of *Cnr2* gene transcripts in rodents treated with opioids, cocaine and alcohol in comparison to control animals. Animals treated with cocaine or heroin showed increased Cnr2 gene transcripts in comparison to controls, indicating the presence of *Cnr2* gene transcripts in the brain that is influenced by abused substances [15-17]. There was also an association between the Q63R polymorphism of the *CNR2* gene and alcoholism in Japanese population. The Q63R polymorphism in the *CNR2* gene has been reported to be associated with autoimmune diseases [42] and with osteoporosis [43]. Although the biologic and genetic mechanisms common between osteoporosis and alcoholism are not well established, heavy alcohol consumption and alcoholism disrupt bone mineral density and increase the incidence of fractures. Little has also been reported on the link between autoimmune disorders and alcoholism. However, one of the least appreciated medical complications of alcohol abuse is altered immune regulation leading to immunodeficiency and autoimmunity [44]. It therefore appears that immunocannabinoid activity in the nervous system may play a role in controlling stress responses and alcohol dependence and CB2-Rs in the brain and may be a novel target to modulate the effects of cannabinoid. Thus, because CNS CB2-Rs and environmental factors, such as stressors, appear to be involved in susceptibility to alcoholism, then CB2-R antagonists may be useful in the treatment of alcoholism.

Our studies provided the first evidence for the CNS effects of CB2-Rs and its possible involvement in drug addiction and neuropsychiatric disorders [15-17, 45, 46]. We utilized behavioral and molecular methods to study and determine whether there was a link between depression that may be a factor in drug/alcohol addiction and CNS CB2-Rs. First we established the use of mouse CMS model of depression which has been validated and a widely used model for screening anti-depressants. Briefly the mouse CMS model measures one of the core symptoms of depression which is anhedonia, a lack of pleasure. Then, mice were subjected daily for four weeks to CMS, and anhedonia was measured by the consumption of sucrose solution. Behavioral and rewarding effects of abused substances were determined in the CMS and controlled animals. The expression of CB2-Rs and their gene transcripts was compared in the brains of CMS and control animals by Western blotting and RT-PCR. CMS induced gender-specific aversions in the test of anxiety which were blocked by WIN55212-2 and CB2-R agonist. In other studies we demonstrated that direct CB2-R antisense oligonucleotide microinjection into the mouse brain induced anxiolysis, indicating that CB2-Rs are functionally present in the brain and may influence behavior [15-17, 45, 46].

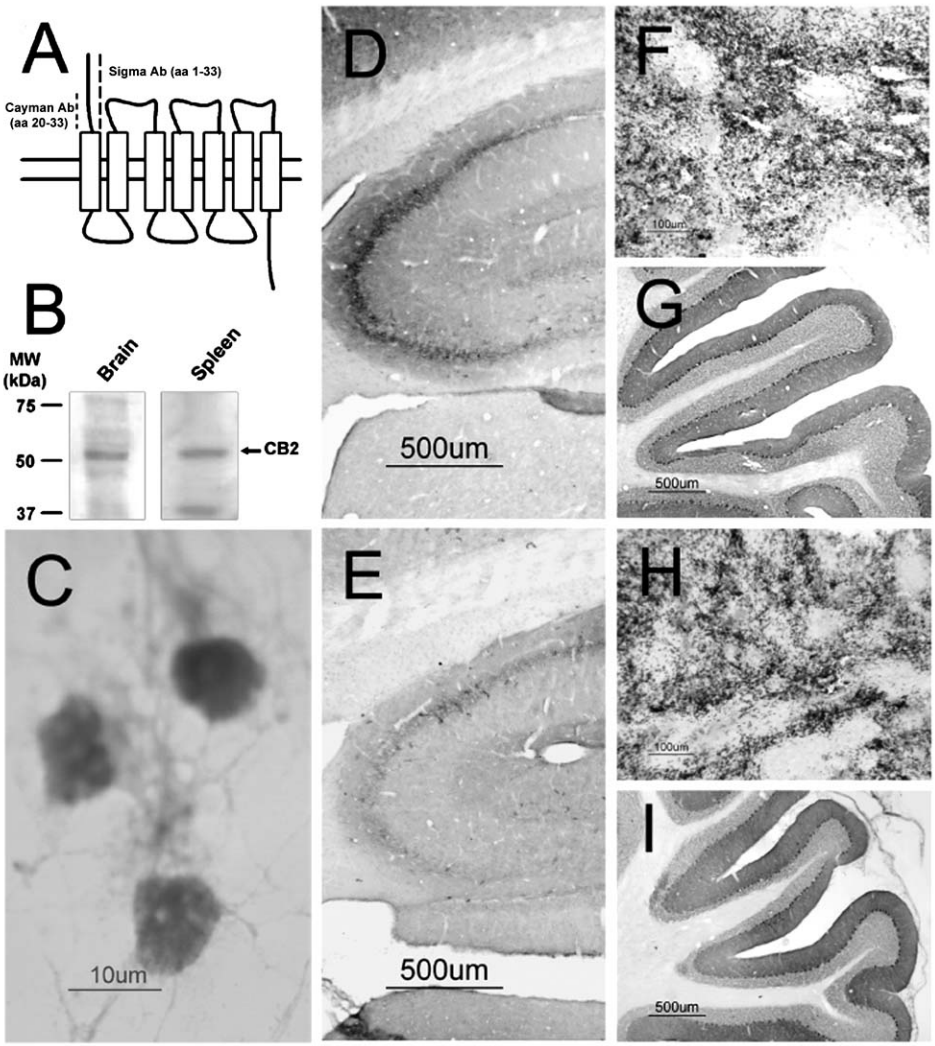

Fig. (3). Characterization and immunohistochemical localization of CB-Rs. (**A**) Diagram showing locations of CB2 epitopes used to generate CB2-R antibodies. (**B**) Western analyses of proteins separated on 4-20% SDS-PAGE gels along with size standards and visualization was with enhanced chemiluminescence. Lysates were prepared from the rat spleen and brain regions as noted. (**C**) Bright-field photomicrograph of CB2 immunostaining in neuronal cells in primary hippocampus culture. (**D**) CB2 immunostaining using CB2-R antibody in a section through the hippocampus CA2 region, with intense staining in pyramidal cells. (**E**) Lack of iCB2 in a section incubated in the presence of the immunizing protein. (**F** to **I**) Demonstrating similar localization patterns in the spleen, (**F**) and (**H**) and cerebellum, (**G**) and (**I**) using two different CB2-R antibodies.

COMMENTS

The clinical and functional implication of neuronal CB2-Rs in the brain will gradually become clearer because more research will certainly unravel the contribution and interaction of CB1 and CB2-Rs in drug addiction and neuropsychiatry. The new knowledge from our data and those of other recent studies that CB2-Rs are present in the brain raises many questions about the possible roles that CB2-Rs may play in the nervous system. These results therefore extend the previous evidence that CB2-Rs are playing an important role in immune function to other putative neuronal function by their apparent presence in neuronal processes. Our studies implicate neuronal and glial CB2-Rs in the chronic mild stress model of depression, and substance abuse. The immunohistochemical localization of CB2-Rs, when compared to that of known CB1-R distribution in the brain, may be an indication of other putative functional roles of CB2-Rs in the CNS. Therefore both CB1 and CB2 receptors seem likely to work both independently and/or cooperatively in differing neuronal populations to regulate important physiological activities in the central nervous system. Recent events in the clinic have linked the use of the anti-obesity drug, Accomplia, a CB1-R antagonist and an appetite suppressant with a higher risk of depression and suicide. Our data have also showed associations of the *CNR2* gene with depression in a human population and also in the CMS model of depression [17, 45] suggesting that the CB2-Rs may be involved in the endocannabinoid signaling mechanisms associated with the regulation of emotionality. More studies are therefore required to determine if CB2-R ligands have the risk of depression or suicide that has led to the withdrawal of

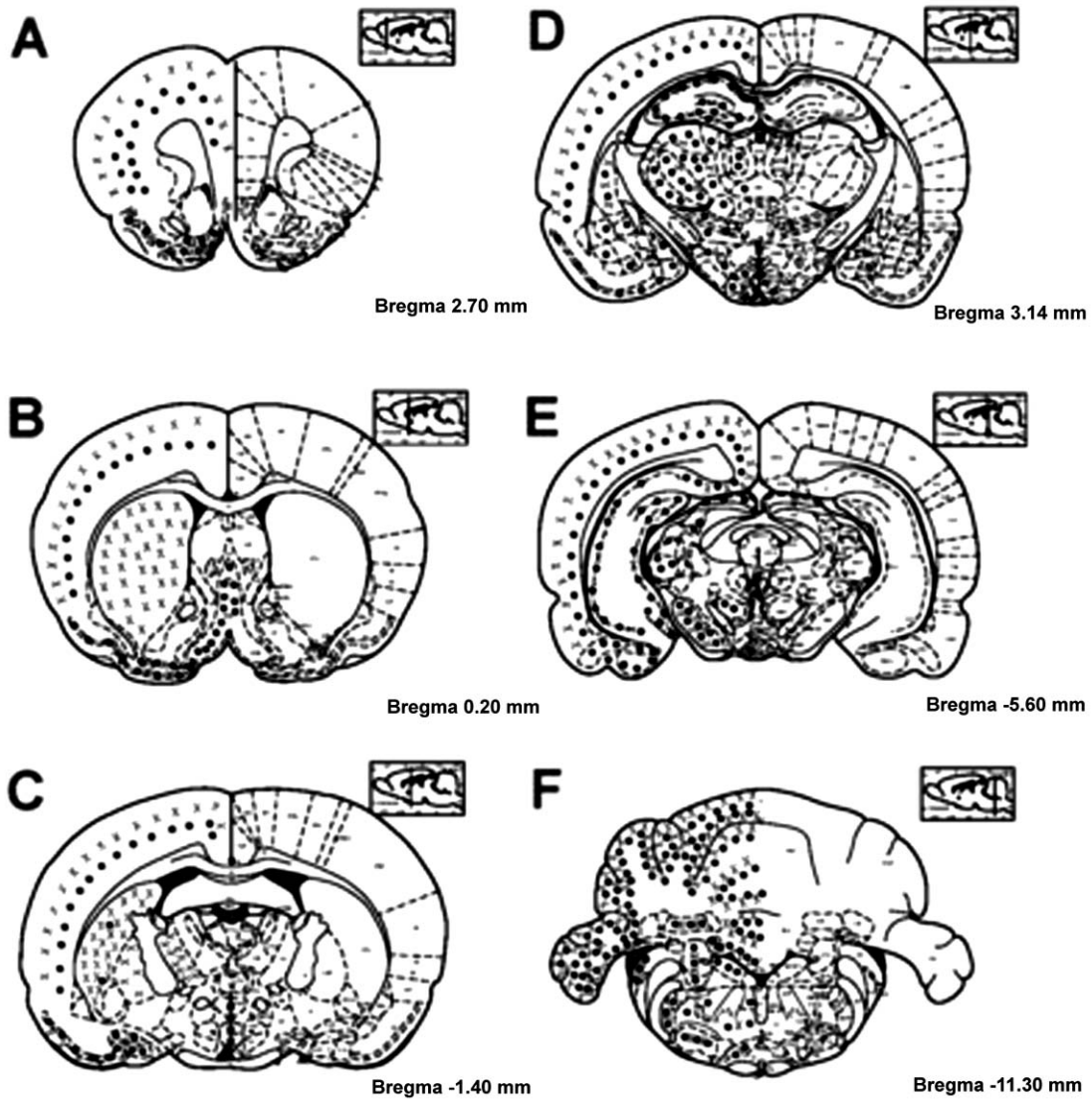

Fig. (4). Schematic representation of CB2-immunopositive structures in the rat brain corresponding to rostrocaudal levels with respect to bregma. Closed circles indicate immunopositive perikarya. X represents immunoreactive fibers and nerve terminals.

rimonabant from use as an appetite suppressant in the control of obesity in Europe.

ACKNOWLEDGEMENT

ESO acknowledges financial support from William Paterson University center for research, the Dean, Dr. Sandra DeYoung for continued student worker support and the Provost office for release time. Dr. Uhl's MNB branch is supported by NIDA-NIH. HI acknowledges financial support from Imai Kimi memorial research fund for stress related disorders.

ABBREVIATIONS

CB2-R = Cannabinoid receptor 2

CB1-R = Cannabinoid receptor 1

CBR = Cannabinoid receptor

CBRs = Cannabinoid receptors

eCBs = Endocannabinoids

ECS = Endocannabinoid system

REFERENCES

[1] Onaivi ES, Sugiura T, Di Marzo V, Eds. Endocannabinoids: the brain and body's marijuana and beyond. Boca Raton: CRC Taylor and Francis 2006.

[2] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993; 365: 61-5.

[3] Galiegue S, Mary S, Marchand J, *et al*. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995; 232: 54-61.

[4] Griffin G, Wray EJ, Tao Q, *et al*. Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for CB2 receptor absence in the rat central nervous system. Eur J Pharmacol 1999; 377: 117-25.

[5] Benito C, Nunez E, Tolon RM, *et al.* Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. J Neurosci 2003; 23: 11136-41.

[6] Ibrahim M, Deng H, Zvonok K, *et al.* Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain

inhibition by receptors not present in the brain. PNAS 2003; 100: 10529-33.
[7] Golech SA, McCarron, RM, Chen Y, *et al*. Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. Mol Brain Res 2004; 132: 87-92.
[8] Nunez E, Benito C, Pazos MR, *et al.* Cannabinoid CB2 receptors are expressed by perivascular microglia cells in the human brain: An immunohistochemical study. Synapse 2004; 53: 208-213.
[9] Benito C, Kim WK, Chavarria I, *et al.* A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis. J Neurosci 2005; 25: 2530-6.
[10] Sheng WS, Hu S, Min X, *et al.* Synthetic cannabinoid WIN55212-2 inhibits generation of inflammatory mediators by IL-IB-stimulated human astrocytes. Glia 2005; 49: 211-9.
[11] Palazuelos J, Aguado T, Egia A, *et al*. Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. FASEB J 2006; 20: E1773-9.
[12] Brusco A, Tagliaferro P, Saez T, *et al.* Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. Synapse 2008; 62: 944-9.
[13] Chin CL, Tovcimak AE, Hradil VP, *et al*. Differential effects of cannabinoid receptor agonists on regional brain activity using pharmacological MRI. Br J Pharmacol 2008; 153: 367-79.
[14] Viscomi MT, Oddi S, Latini L, *et al*. Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the P13K/Akt pathway. J Neurosci 2009; 29: 4564-70.
[15] Onaivi ES. Neuropsychopharmacological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. Neuropsychobiology 2006; 54: 231-46.
[16] Ishiguro H, Iwasaki S, Teasenfitz L, *et al.* Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. Pharmacogenomics J 2007; 7; 380-5.
[17] Onaivi ES, Ishiguro H, Gong J-P, *et al*. Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. Ann NY Acad Sci 2008; 1130: 434-49.
[18] Pazos MR, Nunez E, Benito C, *et al.* Role of the endocannabinoid system in Alzheimer's disease: new perspectives. Life Sci 2004; 75: 1907-15.
[19] Benito C, Tolon RM, Pazos MR, *et al*. Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol 2008: 153: 277-85.
[20] Paldyova E, Bereczki E, Santha M, *et al*. Oladin ether, a putative endocannabinoid, inhibits mu-opoid receptor activation *via* CB2 cannabinoid receptors. Neurochem Int 2008; 52: 321-8.
[21] Paldy E, Bereczki E, Santha M, *et al*. CB2 cannabinoid receptor antagonist SR144528 decreases mu-opioid receptor expression and activation in mouse brainstem: role of CB2 receptor in pain. Neurochem. Int 2008; 53: 309-16.
[22] Onaivi ES, Ishiguro H, Gong JP, *et al.* Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann NY Acad Sci 2006; 1074: 514-36.
[23] Fernandez-Ruiz J, Romero J, Velasco G, *et al.* Cannabinoid CB2 receptor: a new target for controlling neural cell survival. Trends Pharmacol Sci 2006; 28: 39-45.
[24] Van Sickle MD, Duncan M, Kingsley PJ, *et al.* Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 2005; 310: 329-32.
[25] Gong J-P, Onaivi Es, Ishiguro H, *et al*. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. Brain Res 2006; 1071: 10-23.
[26] Suarez J, Bermudez-Silva FJ, Mackie K, *et al.* Immunohistochemical description of the endogenous cannabinoid system in the rat cerebellum and functionally related nuclei. J Comp Neurol 2008; 509: 400-21.
[27] Suarez J, Llorente R, Romero-Zerbo SY, Mateos B, *et al*. Early maternal deprivation induces gender-dependent changes on the expression of hippocampal CB1 and CB2 cannabinoid receptors of neonatal rats. Hippocampus 2009; 19(7): 623-32.
[28] Koch M, Habazetti I, Dehghani F, *et al*. The rat pineal gland comprises an endocannabinoid system. J Pineal Res 2008; 45: 351-60.
[29] Brown SM, Wager-Miller J, Mackie K. Cloning and molecular characterization of rat CB2 cannabinoid receptor. Biochim Biophys Acta 2002; 1576: 277-85.
[30] Shire D, Calandra B, Rinaldi-Carmona M, *et al*. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta 1996; 1307: 132-6.
[31] Abood ME. Molecular biology of cannabinoid receptors. Handb Exp Pharmacol 2005; 81-115.
[32] Quing-Rong L, Chun-Hong P, Akitoyo H, *et al*. Species differences in cannabinoid receptor 2 (CNR2 gene): Identification of novel human and rodent CB2 isoforms, differential tissue expression, and regulation by cannabinoid receptor ligands. Gene Brain Behav 2009; in press.
[33] Grimsey NL, Goodfellow CE, Scotter EL, *et al.* Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies are not all created equal. J Neurosci Methods 2008; 171: 78-86.
[34] Buckley NE, McCoy KL, Mezey E, *et al.* Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB2 receptor. Eur J Pharmacol 2000; 396: 141-9.
[35] Brusco A, Taglaiferro PA, Saez T, *et al.* Ultrastructural localization of neuronal brain CB2 cannabinoid receptors. Ann NY Acad Sci 2008; 1139: 450-7.
[36] Ong WY, Mackie K. A light and electron microscopic study of the CB1 cannabinoid receptor in primate spinal cord. J Neurocytol 1999; 28: 39-45.
[37] Baek J-H, Zheng Y, Darlington CL, *et al*. Cannabinoid CB2 receptor expression in the rat brainstem cochlear and vestibular nuclei. Acta Oto-Laryngol 2008; 128: 961-7.
[38] Ashton JC, Friberg D, Darlington CL, *et al*. Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. Neurosc Lett 2006; 396: 113-6.
[39] Onaivi ES, Carpio O, Ishiguro H, *et al*. Behavioral effects of CB2 cannabinoid receptor activation and its influence on food and alcohol consumption. Ann NY Acad Sci 2008; 1139: 426-33.
[40] Vinod KY, Hungund BL. Endocannabinoid lipids and mediated system: Implication for alcoholism and neuropsychiatric disorders. Life Sci 2005; 77: 1569-83.
[41] Basavarajappa BS, Hungund BL. Role of the endocannabinoid system in the development of tolerance to alcohol. Alcohol Alcohol 2005; 40: 15-24.
[42] Sipe JC, Arbour N, Gerber A, *et al*. Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders. J Leukoc Biol 2005; 78: 231-8.
[43] Karsak M, Cohen-Solal M, Freudenberg J, *et al*. The cannabinoid receptor type 2 (CNR2) gene is associated with human osteoporosis. Hum Mol Genet 2005; 14: 3389-96.
[44] Cook RT. Alcohol abuse, alcoholism, and damage to the immune system –a review. Alcohol Clin Exp Res 1998; 22: 1927-1942.
[45] Onaivi ES, Ishiguro H, Patel S, *et al.* Methods to study the behavioral effects and expression of CB2 cannabinoid receptors and its gene transcripts in chronic mild stress model of depression. In: Onaivi ES, Ed. Marijuana and cannabinoid research: methods and protocols. Totowa: Humana press Inc. 2006; pp. 291-8.
[46] Uhl GR, Ishiguro H, Onaivi ES, *et al.* Molecular neurobiological methods in marijuana-cannabinoid research. In: Onaivi ES, Ed. Marijuana and cannabinoid research: methods and protocols. Totowa: Humana press Inc. 2006; pp. 1-17.

Received: April 17, 2009 Revised: May 12, 2009 Accepted: May 18, 2009