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Endocannabinoid system and CNR1 gene polymorphisms in schizophrenia and addictive disorders

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Substance abuse is the most prevalent comorbid psychiatric condition associated with schizophrenia. Cannabis is a drug frequently used for schizoprenic patients. In the last decades the endocannabinoid system and their endogenous ligands have been discovered. Endogenous cannabinoids act in the brain on cannabinoid CB1 receptor. On the other hand this system may be involved in several brain functions through neuromodulation dopaminergic and other neurotransmitter system involved in schizophrenic and substance abuse disorders. Advances of genetic research have addressed the focus on the search of candidate genes for both disorders. In this review we have summarized the studies published about the CNR1 gene on szhizophrenia and substance abuse disorders.

Key words:
Cannabis. CNR1. Schizophrenia. Addictions.

Actas Esp Psiquiatr 2007;35(2):122-129

Sistema endocannabinoide y polimorfismos del gen CNR1 en la esquizofrenia y los trastornos adictivos

La esquizofrenia presenta una alta comorbilidad con los trastornos adictivos, siendo el cannabis una de las sustancias más frecuentemente consumidas por estos pacientes. En los últimos años hemos asistido al descubrimiento del sistema endocannabinoide, conociéndose que las acciones centrales de los endocannabinoides se vehiculan a través de los receptores cerebrales de tipo 1 (CB1) codificados por el gen CNR1. El sistema endocannabinoide interviene en una gran cantidad de procesos cerebrales a través de su interacción con otros sistemas de neurotransmisión, encontrándose implicado a su vez en la neurobiología de la esquizofrenia y de los trastornos adictivos. La investigación genética en los últimos años se ha dirigido en pacientes con estos trastornos psiquiátricos al estudio de marcadores polimórficos de genes candidatos, entre ellos el gen CNR1. En esta revisión se describen los estudios realizados con el gen CNR1 en ambos trastornos.

Palabras clave:
Cannabis. CNR1. Esquizofrenia. Adicciones.

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ENDOCANNABINOID SYSTEM

Cannabinoid system action method

The term cannabinoid describes a group of compounds that have a carbocyclic structure with 21 carbons, among which its analogues and products from its transformation are included. Among the constitutens of *Cannabis sativa*, more than sixty different cannabinoids have been described. Among these, that having the greatest psychoactive potency is the Δ^9-tetrahydrocannabinol (Δ^9-THC or THC), so that it has been widely studied regarding its action on the brain. Other cannabinoids present in the plant and that have also been studied are: Δ^8-THC, which has a very similar pharmacological profile to that of Δ^9-THC, but only appears in some varieties of the plant and its concentration is very small in comparison with that of Δ^9-THC. The (–) trans-isomers of Δ^9-THC and Δ^8-THC are those that normally appear in the plant[1]. Cannabiol (CBN) also has psychoactive properties, among which those related with discriminative stimuli of THC are found[2]. Its psychoactive activity in animals represents approximately one tenth of that described for THC. However, the results obtained in humans are quite contradictory. Cannabidiol (CBD) is a cannabinoid that has practically no psychoactive properties. Among the pharmacological characteristics attributed to THC, the only one that seems to be shared with CBD is its anticonvulsive activity, although the action mechanisms may be different. Recently a neuroprotector role has been attributed to CBD, on verifying its actions as an antioxidant againts oxidative effects produced in the neurons by glutamate release[3]. CBD has been related with the immune system for some time, it having been observed that it suppresses the production of antibodies in mouse spleen cell cultures[4].

For a long time, the hydrophobic properties of exogenous cannabinoids suggested that their action mechanism would be produced through interaction with the lipid components of the cell membrane, so that they would disorganize the lipid phase, producing an increase in plasma membrane flow[5]. This idea changed after the pharmacological characterization of the CB1 receptor in 1988 in the brain and peripheral nerves of different animals (rat, monkey, guinea pig)[6] and in humans[7] and of the CB2 receptor in 1993 in the rat spleen and in a human leukemic line.[8]

The discovery of these receptors stimulated research in search of its natural ligand. This substance was isolated in the pig brain in 1992[9], verifying that its chemical structure differed from the cannabinoids extracted from *Cannabis sativa.* Its structure corresponds to a derivative of fatty acids related with prostaglandins. This endogenous ligand was called *anandamide* and has high affinity for the CBI receptors and shares many of the THC actions. It has been identified in the human and rat brain and peripheral tissues. Since then, other similar endogenous ligands, such as 2-arachidonoyl glycerol[10,11] and 2-arachidonoyl glyceryl ether have been isolated[12]. Both endocannabinoids produce the same effects in mice as THC, but their intensity is less. This indicates that they could act as partial agonists of the receptor. Other substances related with anandamide, that have behaved as partial agonists of CB1, but with less efficacy, are homo-γ-linolenylethanolamide and 7,10,13,16-docosatetraenylethanolamide[13]. This suggests the possibility of the existence of an endocannabinoid system with a greater number of receptors and endogenous ligands related with anandamide. The cannabinoids exert their effects through interaction with their specific CBI and CB2 receptors.

It seems that both anandamide and its receptors are located in the neuronal lipid membrane and act as neuromodulators through intracellular G proteins, controlling the cAMP and exchange of Ca^{2+} and K^+ ions[14,15] (fig. 1). This system may have important interactions with other neurotransmitters, including gabaergic[16], opioid[17-19] monoaminergic[20,21], glutamatergic[22-24], colinergic[25] systems and adrenal pituitary axis[26,27]. Specifically, TCH measures increase of dopamine in the nucleus accumbens and prefrontal cortex[28,29]. This effect, which is shared with other substances that may be abused, could constitute the substrate of

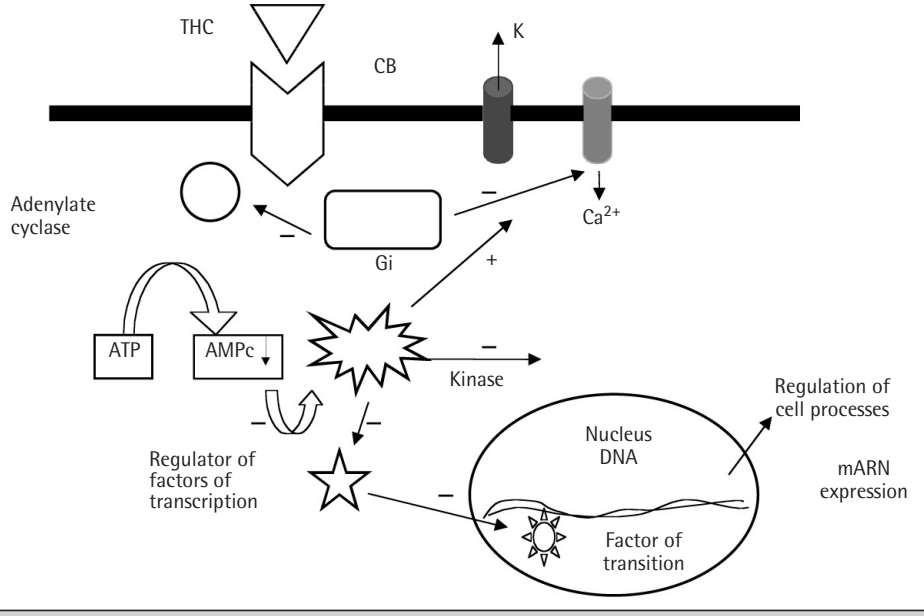

Figure 1 *Action mechanism of CB1 receptor. The cannabinoid type (CB1) receptor has a protein nature, it being coupled to adenylatecyclase by a Gi protein. Thus, the action of the cannabinoids generally leads to decrease of cAMP levels. This cyclic nucleotide activates some kinases, the fall of their levels will decrease phosphorylation of the substrates of these enzymes. This is the case of some K^+ channels, that are phosphorylated by these kinases, their dephosphorylation leads to an increase of conductance. This decreases depolarization of the membrane and reduces neurotransmitter release present in the presynaptic terminal. Other cAMP dependent kinases participate in the modification of genetic expression, on acting on other transcription factors, that regulate mRNA expression for certain proteins. Use of the cellular transcription mechanimsm by the cannabinoids may produce a modification of genetic expression, which could lead to «chronification» of certain metabolic activities. Modified from Ramos Atance JA and Fernández Ruiz J, 1999. THC: tetrahydronanabinol receptor; CB: cannabinoid receptor; K^+: potassium ion; CA^{2+}: calcium ion; Gi: Gi protein; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; mRNA: messenger ribonuclic acid.*

the reinforcing properties and of its use with recreational purposes.

Distribution of the cannabinoid receptors

The CB1 receptor has been located in different regions of the central nervous system, in peripheral terminations of the nerves and in the testicles[30]. In the brain, it is distributed in the cerebral cortex, limbic areas (including hippocampus and amygdala), basal ganglia, cerebellum, thalamus and brain stem)[31-33] (table 1). The distribution of the cannabinoid receptors is well correlated with some of the pharmacological effects produced by these compounds. In practice, it can be considered that all the effects produced by cannabinoids on the central level are mediated by the CB1 receptor.

Anandamide has been identified in the rat and human brain and peripheral tissues. In both species, it is distributed through hippocampus, striatum and cerebellum, CB1 rich regions and in the thalamus where the expression of the receptor is very low. It also appears in the spleen, where there are high levels of the CB2 receptor. Small amounts of anandamide have been detected in the human heart in rat skin and in traces in human serum, plasma and cerebrospinal fluid[34] .

Endocannabinoids do not have the same distribution pattem in the brain as the CB1 receptors[33]. However, there are regions such as the spinal cord and brain stem where the relatively high levels of endocannabinoids contrast with a low density of CBI receptors, or in the cerebellum, where the receptor density is high but the endocannabinoid levels are low. The reason for these differences is not known at present (table 1).

Table 1 Localization of endocannabinoid and CB1 receptors in different cerebral regions

Cerebral region	Anandamide	2-arachidonoyl-glycerol	CB1 receptors
Spinal cord	+++	++++	++
Brain stem	++++	+++++	++
Mesencephalon	++	++	++
Cerebellum	+	++	+++++
Diencephalon	+	+	+++
Hippocampus	+++	+++++	++++
Limbic nuclei	++	++++	++
Striate body	+++	++++	++++
Cerebral cortex	+	++	++

Anandamide (pmoles/g tissue): + (< 20), ++ (20-40), +++ (40-60 >), ++++ (60-80), +++++ (80). 2-AG (nmoles/g tissue): + (< 3), ++ (3-6), +++ (6-9), ++++ (9-12), +++++ (> 12). CB1 receptor (fmol/mg tissue): + (< 250), ++ (250-500), +++ (500-750), ++++ (750-1.000), +++++ (> 1.000). CB1: cannabinoid type 1. Bisogno et al., 1999.

Genomie localization of the CNR1 gene

The CNR1 gene that codes the CB1 receptor is located in chromosome 6 q14-q15[35]. This gene has at least four exons[36]. Recent studies have revealed that there is a differential expression in the central nervous system of five mRNA, the most abundant being the messengers that express exon one. Two alterative sites for the initiation of the transcription located in exon 1 and 3, respectively have also been identified. The initiation sequence of exon 1 transcription has more activity than that located in exon 3. These data are consistent with the relatively high abundance of Cb1A, Cb1B, Cb1C and Cb1D variants, that have exon 1, against the low abundance of the isoform Cb1E for which the initiation site of the exon 3 transcription seems to be used[36].

The CNR1 gene has several polymorphisms over all the sequence. Many of them have been used, as we will see below, for the study of the implication of the endocannabinoid system in several diseases.

CANNABINOID SYSTEM AND SCHIZOPHRENIA

Psychological responses to cannabis

It is known that consumption of cannabis produces adverse mental effects in a high proportion of consumers. Many of these effects are dose-dependent, although the adverse symptoms may be worsened by constitutional factors such as age, personality traits and vulnerability to suffer a mental disorder[37]. Theoretically, cannabis may precipitate psychosis in different ways, from pictures of self-limited toxic organic psychosis to pictures of chronic psychoses with maintained consumption. It can be stated that its use could constitute a risk factor for major psychiatric disorder such as schizophrenia[38-49].

There is practically a generalized consensus that consumption of cannabis is associated with a greater frequency of psychotic relapses and with exacerbation of schizophrenic symptoms[50], above all in significant consumers, the so-called heavy consumers in medical literature.

Schizophrenia and cannabis consumption

The biological explanation that has been given to the cannabis-schizophrenia relationship is the following: tetrahydrocannabinol (THC) behaves as a dopaminergic agonist that acts on the mesolimbic dopaminergic, tuberoinfundibular and nigrostriatal system[51]. According to the dopaminergie hypothesis, schizophrenia is due to failures in the dopaminergic system and other neurotransmitters

involved[52]. As is known, the antipsychotic effect of neuroleptics is due to the blockage of dopaminergic receptors. This blockage may be counteracted either by a dopamine increase or by the intervention of an agonist such as THC. In this way, cannabis consumption would cause exacerbations or psychotic recurrences. THC unblocks the dopamine receptors in the previously established areas, canceling the neuroleptic action. Mailleaux and Vanderhaeghen[53] describe the upregulation of the cannabinoid receptors in the rat putamen and caudate, mediated by the NMDA receptors. This would indicate a relationship of the cannabinoid system, not only in the mesolimbic dopaminergic system, but also in glutamatergic one.

Another aspect to consider is that cannabis consumption is done to alleviate the extrapyramidal effects of the antipsychotics, but considering that cannabinoids decrease antipsychotic plasma levels, they would cause a psychotogenic effect.

CB1 receptor and schizophrenia

Given the relationships found in the clinical setting between cannabis consumption and schizophrenia, modulation of the cannabinoid system on the neurotransmission systems involved in neurobiology of schizophrenia, especially on the dopaminergic system, and the neuroanatomical relationship existing between cannabinoid receptors and dopaminergic pathways, basic research has been aimed at the study of the relationships between cannabinoid system and schizophrenia.

As we have already mentioned, CB1 receptor is coded by the CNR1 gene[35], two polymorphisms having been studied in schizophrenic patients: SNP 1359G/A[54,55] and microsatellite (AAT)n[56] that is located in the end of 3'UTR of the gene.

The existing studies that have tried to demonstrate the association of the variations of this gene with schizophrenia are described in table 2.

In 1995, Dawson described the first association study conducted in a group of 131 schizophrenics compared to 103 control subjects of the Caucasian population, in which no association was found between polymorphism (AAT)n and schizophrenia[56]. Tsai et al. (2000), in the study conducted in a Chinese population in 127 schizophrenic subjects and 146 controls, also did not find any association between the different alleles of the polymorphism (AAT)n of the CNR1 gene and schizophrenia[57]. In the Leroy et al. series (2001) in the French Caucasian population, polymorphism 13 59/A was studied in 102 subjects with schizophrenia and 63 controls[58]. They did not find any differences in the allelic and genotypal distribution, indicating a tendency to association in the allelic distribution in patients with substance abuse compared to non-consumers. This consisted in a decrease of frequency for the 1359G allele in non-consumers. Ujike et al. (2002) described that the microsatellite (AAT)n, but not the SNP 1359G/A of the CNR1 gene was significantly associated with the hebephrenic subtype in a sample of 121 subjects with schizophrenia compared with 148 controls of the Japanese population[59]. In a recently conducted study (in press), in a sample of 131 schizophrenic subjects and 115 controls, our group found significant differences when comparing the frequencies for alleles of microsatellite (AAT)n when comparing schizophrenics who do not consume abuse substances and control population (data not published). Finally, we must consider that the negative findings in the association of a certain polymorphism may not

Table 2 CNR1 polymorphism studies in schizophrenic subjects

References	Sample	Comments
Dawnson et al., 1995	Caucasian population 131 schizophrenics 103 controls	They find no association between polymorphism (AAT)n and shizophrenia
Tsai et al., 2000	Chinese population 127 schizophrenics 146 controls	They find no association between polymorphism (AAT)n and schizophrenia
Leroy, et al., 2001	Caucasian french population 102 schizophrenics (42 consumers and 60 non-consumers) 63 controls	Polymorphism 1359G/A. is studied. They find less frequency of allele 1359G in the non-consumer group
Ujike et al., 2002	Japanese population 121 schizophrenic patients 148 controls	They describe the fact that polymorphism (AAT)n but not 1359G/A of CNR1 gene is significantly associated with the hebephrenic subtype

exclude a certain locus as a whole, since it is possible that additional variants of this locus may influence the pathogenesis of schizophrenia.

CANNABINOID SYSTEM AND SUBSTANCE ABUSE DISORDERS

Different facts support the contribution of the CNR1 gene in vulnerability to substance abuse disorders. This receptor is widely distributed in brain regions related with reward and with «memory» of consumption, including the hippocampus, caudate nucleus and cerebral cortex[60-62]. A co-localization of the CB1 receptors and dopaminergic ones in the same cerebral synapsis has been observed in relationship with addiction[60]. CB1 receptors measure reward of the active components of marijuana. They also modulate the dopaminergic neurotransmission in the reward brain circuits of other abuse substances[63]. Knockout (KO) mice for this receptor or mice treated with antagonists have less reinforcing effect for difference addictive substances, including cannabinoids, morphine, nicotine and ethanol[63-66].

On the other hand, studies done in subjects affected by different addictions support the contribution of several polymorphisms of the CNR1 gene to vulnerability for substance abuse (table 3).

Table 3 CNR1 polymorphism studies in subjects with addictive disorders

CNR1 polymorphism	Population	Reference
AAT microsatellite	Non-hispanic caucasian	Comings et al., 1997
AAT microsatellite	Caucasian	Jhonson et al., 1997
AAT microsatellite	Chinese	Li et al., 2000
AAT microsatellite	Caucasian	Heller et al., 2001
SNP 1359 G→A		
Thr 453 Thr		
AAT microsatellite	European-american	Covault et al., 2001
	Afro-american	
SNP 1359 G→A; Thr 453 Thr	Caucasian	Schmidt et al., 2002
SNP rs2023239 (hCV116000616)	European-american	Zhang et al., 2004
	Afro-american	
Haplotype TAG (rs806379	European-american	
[hCV1652584] = T,	Afro-american	
rs1535255 [hCV8943758] = A,	Japanese	
rs2023239[hCV11600616) = G	European-american	
Haplotype (rs754387		
(hCV9662507) = C		
and rs180619(hCV15841551) = C)		

The (AAT)n microsatellite of the CNR1 gene has been studied in population groups belonging to different ethnic groups and in populations of patients affected by different addictions, with controversial results. Comings et al. (1997)[67] hypothesized that variants of this gene could be associated with susceptibility to alcohol abuse or drug dependence. In a sample of non-Hispanic Caucasian subjects, they found a significant association between the genotype of long alleles (≥ 5l/≥ 5) of this polymorphism with different types of dependence (cocaine, amphetamines, cannabis) and with the use of intravenous drugs, but not with the variables related with alcohol abuse/dependence. This same group also found a significant association between the number of triplete repetitions and the P300 wave that is found to be related with substance abuse[68]. Li et al. (2000) tried to replicate Comings' findings in a sample of opiate dependent Chinese population, not finding evidence that the polymorphism (AAT)n of the CNR1 gene confers susceptibility to heroin abuse[69]. The study conducted by Covault et al. (2001) in two European-American (EA) and Afro-American (AA) samples dependent on alcohol or drugs versus controls also did not find any differences in allelic frequencies for this polymorphism[70]. Heller et al. (2001), in the study conducted in the Caucasian population addicted to intravenous drugs, studied the polymorphism (AAT)n and SNP 1359G/A. They did not find any association with drug consumption[71]. Schmidt et al. (2002) also studied this SNP in a sample of 121 serious alcoholics from the Caucasian German population and 136 non-alcoholic controls[72]. They found a greater frequency of 1359A in the alcoholic subject group and greater frequency of genotype A/A in subjects with a background of alcoholic delirium, so that they suggested that the presence of the CNR1 1359 A/A genotype could confer vulnerability to delirium in the alcoholic abstinence syndrome. In a study conducted in European-American (EA) (n = 169), Afro-american (AA) (n = 85) polydependent subjects and Japanese dependent on alcohol (n = 285), compared with controls (n = 322, n = 212 and n = 463, respectively), Zhang et al. (2005) found a strong association of the haplotype TAG (rs806379 [hCV1652584] = T, rsl 535255 [hCV8943758] = A, rs2023239 [hCV11600616] = G]) both for the polydependent EA and AA samples and for the alcohol dependent Japanese population sample. This same study also found an association of haplotype 1359A/3813A/4894G of exon 4 in AA subuects versus controls, but not between EA subjects. They also found an association in dependent subjects compared to EA controls, but not in AA ones, with haplotype (rs754387 [hCV9662507] = C and rsl 80619 [hCV1584155 1] = C) located in region 5' of the CNR1 gene. This same study also describes an association of allelic frequencies in dependent subjects for both EA and AA populations for the polymorphism rs2023239 (hCVl 16000616) in the end 3' of intron 2 of this same gene.

This study is an example of how the use of haplotypes may increase the statistical analysis power and the consequent detection of associations between variations of a gene and a certain phenotype or trial. In any event, CB1 is one of the most abundant neuromodulator receptors in the central nervous system and the genotype/phenotype relationships observed may be due to different levels/pattems

of expression which, in tum, would alter the dopamine levels in the synapsis.

Data from our group (unpublished data) in which 58 non-consuming schizophrenic subjects compared to 78 polyconsumers in a Spanish population were compared do not find any association between the polymorphism (AAT)n type microsatellite and substance dependence.

CONCLUSIONS

Thus, the relationships found up to now between the endocannabinoid system and schizophrenia suggest that there could be a dysfunction of this system in at least some subtypes of schizophrenia. On the other hand, the association of the CNR1 gene variants with subjects dependent on different substances also seems to be demonstrated. This fact could be related with the possibility that the implication of the CNR1 gene in vulnerability to addiction is common to the substances susceptible to abuse. Finally, the fact that the endocannabinoid system has a neuromodulating action on dopaminergic, involved both in the neurobiology of schizophrenia as well as the addictions, would support the hypothesis that, at least in some schizophrenic subjects vulnerable to consumption of substances would have genetic differences related with variations of the CNR1 gene, that confers greater susceptibility to abuse.

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