The Implication of CNR1 Gene’s Polymorphisms in the Modulation of Endocannabinoid System Effects

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The endocannabinoid system (ECS) represents one of the most important physiologic systems involved in organism homeostasis, having various implications upon individual behavior and metabolic phenotype. It is composed of cannabinoid receptors CB1 and CB2, and their genes (CNR1 and CNR2), their endogenous ligands and the enzymes which mediate endogenous ligands’ biosynthesis and degradation. Anandamide and 2-arachidonoylglycerol are two endogenous agonists of the cannabinoid receptors. It is considered that ECS connects physical and emotional response to stress with appetite and energy balance, functioning like an after stress recovery system which remains inactive in repose physiologic conditions. It is involved in several physiologic processes like nociception, motor control, memory, learning, appetite, food intake and energy balance. This review analyzes the implication of 11 polymorphisms of CNR1 gene in the modulation of the ECS metabolic and central effects. A lot of studies show that rs12720071, rs1049353, rs806381, rs10485170, rs6454674, rs2023239 polymorphisms are associated with metabolic effects. From them rs12720071, rs104935, rs6454674, rs2023239 polymorphisms are also associated with central effects of ECS (substance addiction, impulsivity, resistance to antidepressive treatment). Other studies indicate that rs806368, rs1535255, (AAT)9,(AAT)12 and (AAT)n are correlated only with central effects (schizophrenia, substance addiction, impulsivity, Parkinson syndrome). The discovery of ECS and its signaling pathways opens a door towards the understanding of several important physiologic processes regarding appetite, food intake, metabolism, weight gain, motor control, memory, learning, drug addiction and nociception. The detailed analysis and validation of the ECS functioning can bring us very close to the discovery of new diagnosis and treatment methods for obesity, drugs abuse and numerous psychic diseases.

Key words: endocannabinoid system, CNR1 gene, CNR1 polymorphism, CB1 receptors, metabolic phenotype, central effects.

The endocannabinoid system (ECS) represents one of the most important physiologic systems involved in organism homeostasis, having various implications upon individual behavior and metabolic phenotype.

This system has several structural and physiologic characteristics. The history of the ECS discovery is very special. Although the psychoactive effects of Cannabis sativa plant were known also in antiquity, the basic active Cannabis component – Δ9-tetrahydrocannabinol (THC) – was identified only in 1964 [1]. In 1988 Howlett et al. discovered the receptors with high affinity for cannabinoids. Cannabinoid receptor 1 and 2 (CB1 and CB2) receptors were cloned later. Taking into consideration the presence of cannabinoid receptors, one has tried to determine the endogenous ligands of these receptors. Anandamide (N-arachidonylthanolamine) and 2-arachidonoylglycerol (2-AG) are the first endogenous cannabinoids discovered.

THE STRUCTURE OF ECS

ECS is a complex system with multiple roles in organism. It is composed of cannabinoid receptors CB1 and CB2, their endogenous ligands and the enzymes which mediate endogenous ligands’ biosynthesis and degradation [2].

CB receptors belong to a big receptor family and are G protein-coupled receptors [3]. CNR1 and CNR2 are the genes which codify the cannabinoid receptors CB1 and CB2, being localized at 6q14-q15 level for CNR1 and 1p35-p36.1 level for CNR2. Endocannabinoid receptors are expressed mainly in the brain, but also in other organs involved in energetic homeostasis: adipose tissue, ROM. J. INTERN. MED., 2009, 47, 1, 9–18
liver, gastrointestinal tract, pancreas and skeletal muscle [4][5][6]. CB1 receptor is the most frequent G protein-coupled receptor in the brain [4]. In the central nervous system there have been observed increased CB1 receptor densities in basal ganglia (globus pallidus, putamen, black substance) and in the cerebellum; these positions justify the ECS implication in locomotory activity. The increased level of CB1 expression in neocortical areas and hippocampus can be correlated with cannabinoids’ effects on memory and cognitive function. The low expression of CB1 in spinal cord and brainstem explains the low effects of cannabinoids upon cardiovascular and respiratory functions [7–9].

In the brain the receptor is predominantly expressed in the hypothalamus and at the level of pituitary gland and its activity modulates hypothalamic-pituitary-adrenal axis [4]. In the hypothalamus, CB1 receptor is an important component of neuronal circuits involved in appetite and caloric intake control. It is considered that CB1 receptors’ stimulation at the level of appetite modulator centers determines preferential ingestion of palatable food items.

CB2 cannabinoid receptor is expressed at the level of immune cells (B lymphocytes, T lymphocytes and monocytes), spleen and tonsils, indicating their implication in immune functions [5]. In case of an inflammatory status, CB2 receptors can be expressed also in microglial cells of the brain [11].

Recent studies on mice with CB1 and CB2 deletion indicated the existence of supplementary endocannabinoid receptors non-CB1 and non-CB2 in the brain [12][13].

ENDOCANNABINOID BIOSYNTHESIS

Anandamide is synthesized at the level of nervous tissue through a condensation reaction ATP-independent of arachidonic acid and ethanolamine [17][18]. A lot of studies have shown that this reaction is catalyzed by the reverse action of fatty acid amide hydrolase (FAAH), an enzyme whose direct action is to hydrolyze anandamide [19]. But this enzyme needs concentrations of anandamide precursors superior to those existing in physiologic conditions at the cellular level. That is why it is less probable for this enzyme to have a role in anandamide synthesis in physiologic conditions [20]. Another model of anandamide biosynthesis is represented by phospholipid precursor hydrolysis, N-arachidonoyl phosphatidyl-ethanolamine (PE), catalyzed by phospholipase D [21–23]. At the neuronal level there are two possibilities for 2-AG biosynthesis to occur: phospholipase C mediated hydrolysis of membrane phospholipids resulting diacylglycerol, which is converted to 2-AG by diacylglycerol lipase (DAGL); alternatively phospholipase A1 can generate lisophospholipid consequently hydrolyzed by lisophospholipase C [24].

Both anandamide and 2-AG are generated and released at neuronal level through a mechanism which does not imply vesicular secretion [24]. Having different synthesis pathways, anandamide and 2-AG seem to have also different synthesis and releasing stimuli. It has been observed that activation of dopaminergic D2 receptors in striate increases anandamide and not 2-AG eliberation [25], while the activation of N-methyl-D-aspartate receptors (NMDA) by glutamine in cortical neurons increases 2-AG level, but it does not have any effect on anandamide [26].

ENDOCANNABINOID DEGRADATION

Anandamide hydrolysis is mediated by FAAH resulting ethanolamine and free fatty acid [27]. FAAH is a hydrolytic enzyme for both anandamide and 2-AG, but in high concentration [28–30]. Recent studies have shown that FAAH can also have a reverse action, mediating anandamide synthesis from arachidonic acid and ethanolamine [17][18]. 2-AG hydrolysis in fatty acid and glyc erol is mediated by monoacylglycerol lipase [31].
PHYSIOLOGIC EFFECTS OF ECS
(Fig. 1)

ECS seems to be present in all vertebrates and in some nonvertebrate species, but with some small differences in receptors’ structure and activity, which indicate their implication in vital biologic processes [4][32][33].

It is considered that ECS connects physical and emotional response to stress with appetite and energy balance, functioning like an after stress recovery system which remains inactive in repose physiologic conditions [3]. It is involved in several physiologic processes like nociception, motor control, memory, learning, appetite, food intake and energy balance [4][34][35].

At neuronal level, the depolarization of postsynaptic membranes leads to the de novo synthesis of 2-AG and anandamide through phospholipid dependent pathways [32][36]. Endocannabinoid synthesis as response to membrane depolarization depends on intracellular calcium increment [35][37][38]. Endocannabinoids are released in the synaptic gap and bind to CB1 receptor; this binding has a role in blocking neuromediator release which led to their synthesis and release (dopamine, GABA, glutamate) [32][36]. Endocannabinoids appear to be synthesized “on demand” where and when they are needed [36]. At peripheral level endocannabinoids are also synthesized “on demand” and act in a paracrine or autocrine manner [36].

Cannabinoid receptors’ activation stimulates hunger and increases appetite, especially for sweets and palatable food items [4]. In mice, endocannabinoids levels from the brain rise shortly after food deprivation [39]. Also CB1 receptors are expressed at the level of mesolimbic dopaminergic reward circuits where the perceptions associated with pleasure and appetite stimuli are being processed [4]. Cannabinoid receptors’ agonists have antiemesis effect. Both high and low endocannabinoid levels were associated with mood disorders. The majority of preclinical studies have
shown that CB1 receptors blockade at central level is associated with anxious and depressive states [40–42]. Through CB1 receptors blockade (and probably also CB2) analgesic effects have been observed [43–45]. ECS participates in the reward process, but also in substance addiction effects (alcohol, opioids, nicotine) [46][47]. Endocannabinoids seem to contribute also to neuroprotection, high endocannabinoid levels are found in cerebral vascular accident, cerebral traumatisms, but also in some degenerative diseases such as Parkinson disease, Alzheimer syndrome and multiple sclerosis [46][48][49].

ECS seems to have a role in stimulating relaxation and rest, inducing, after a stress episode, forget of unpleasant memories and food ingestion stimulation. CB1 receptors’ activation seems to have an anxiolytic effect [50][51]. ECS is involved in balance regulation at synaptic level both on long and short term, suppressing both excitatory and inhibitory neurotransmission [4]. ECS induced hunger is considered to be the need to refresh the energy supplies after the stress episode.

ECS regulates organism’s energy balance and peripheral metabolism. Comparing mice with CB1 receptors deletion to the wild-type (with these receptors intact), one could observe that, under the same diet, mice without CB1 tend to be leaner and less hungry than wild-type mice [3]. This suggests the implication of CB1 receptors – endogenous factor – in weight control.

Hepatic CB1 receptors blockade is associated with decreased hepatic expression of the transcription factor SREBP-1c (sterol regulatory element-binding protein) and of its target lipogenic enzymes (acetyl coenzyme-A carboxylase-1 and fatty acid synthase) at mice and attenuates synthesis de novo of hepatic fatty acids in mice with hyperlipidic diet [52]. Hepatic CB1 receptors activation inhibits β-fatty acid oxidation [81][82]. CB1 receptor blockade increased adiponectin secretion from adipose tissue in obese or non-obese mice [52–54]. Matias et al. showed that stimulation of adipocytes CB1 receptors stimulates adipocyte differentiation and lipogenesis [58]. Preclinical data indicate that CB1 receptor blockade may produce increased glucose uptake at the adipocyte level [85]. Cota et al. showed that stimulation of mice adipocytes CB1 receptor increased lipoprotein lipase activity [3].

At metabolic level, CB1 receptors activation increases hepatic lipogenic enzymes expression in mice, while their suppression reduces these enzymes and attenuates synthesis de novo of hepatic fatty acids in mice with hyperlipidic diet. CB1 receptors suppression increases adiponectin level from adipose tissue in obese or non-obese mice [52–54]. The studies show that at the level of glucidic metabolism, CB1 receptors suppression ameliorates glycemic alteration in mice with diet induced obesity, muscle glucose uptake and in humans it reduces HbA1c in patients with type 2 Diabetes Mellitus who present high circulating endocannabinoids levels [55–59].

At the pancreatic β cells level, in vitro pharmacological activation of the CB1 receptors stimulated insulin secretion [10][58][86]. CB1 receptors activation at the gastrointestinal tract level decreased intestinal motility and gastric emptying [83] and increased orexigenic effect of ghrelin [84].

**CNR1 GENE’S POLYMORPHISMS AND WEIGHT GAIN**

Considering the physiologic effects of ECS and the great differences between individuals, many scientists consider that the variations of CB1 receptor’s gene lead to obesity, adipose tissue distribution, metabolic alteration [60], but also various psychic disorders such as schizophrenia, depression, anxiety and drug addiction. More and more studies aim to discover and analyze the various polymorphisms of this gene, in order to anticipate and prevent several diseases based on ECS through CB1 variants.

In a study carried out on a group of European men, Paola Russo analyzed two variants of exon 4 of CNR1, scanning the gene for polymorphisms rs12720071 (3813A/G) and rs806368 (4895A/G). One could observe that allele 3813G was associated with the growth of abdominal circumference (AC), subscapular cutaneous skinfold and body mass index (BMI). Concerning rs806368 polymorphism, there have been observed no associations between these genotypes and the determined variables. The haplotype’s analysis consisted in the studying of 3 frequent haplotypes: A3813A4895 (AA), A3813G4895 (AG), and G3813G4895 (GG), haplotype GG was associated with the increase of the abdominal circumference and subscapular cutaneous skinfold [60].
In another study, the polymorphism rs1049353 (1422A/G) was associated in men with a significant increase of the abdominal circumference, waist to hip ratio and of adipose mass after the adjustment for age and BMI [61]. Adipose mass percent presented a significant association which disappeared after the adjustment for age and BMI [61].

Another study carried out on Swedish and Danish subjects indicates the fact that polymorphisms rs806381 and rs2023239 from the introns level associate with an increased BMI. Continuing the analysis of polymorphisms, the same group of researchers shows two polymorphisms that associate better with an increased BMI: rs6454674 and rs10485170 [62].

Muller et al. study 8 polymorphisms in German children and adolescents: in region 5’ (rs9353527, rs754387, rs6454676), in intron 2 (rs806379, rs1535255), exon 3 (rs2023239), intron 3 (rs806370) and in coding region (rs1049353), but they could not find any link to obesity [63].

THE IMPLICATION OF CNR1 GENE POLYMORPHISMS IN PSYCHIC DISEASES

The connection between ECS and affective state is very well known. The predominance of CB1 receptors in the brain areas responsible with affective state and psychic processes represents a proof for ECS participation in these processes. Numerous studies show the connection between several CNR1 gene’s polymorphisms and psychic diseases, especially hebephrenic schizophrenia. Hebephrenic schizophrenia appears at a very young age, most frequently at puberty or adolescence and has several polymorphic symptoms: oscillatory and childish behavior, with tendency towards antisocial and bizarre acts. The affective disorders are characterized by several aspects: the patient can switch quickly from a usually unmotivated good-humored state to a bad mood, irritability and even lamentation [64].

In a study carried out on Japanese population, Ujike et al. show that the repetition of AAT triplet in region 3’ can be associated with hebephrenic schizophrenia. Rs1049353 (1359G/A) polymorphism in codon 453 cannot be connected to this disease [64][65], but it is associated with resistance to antidepressive treatment [69]. Subjects who present a nine fold repetition of AAT triplet have a 2.3 times higher risk to develop schizophrenia [64].

The same (AAT)n repetition can be observed also in Spanish population [66]. The same polymorphism seems to be present at different peoples in persons with schizophrenia [67][68], Parkinson disease [80].

Hamdan et al. analyzed rs1049353 polymorphism and could not find any connection with the risk to develop schizophrenia, but only with treatment responsiveness, allele G being frequent in patients who do not respond to treatment [69]. In a study made on German subjects, Seifert et al. could not find any connection between the polymorphisms rs6454674, rs1049353, rs136096 and schizophrenia [70].

ALCOHOL AND DRUG ADDICTION RISK

CNR1 gene’s polymorphisms seem to control alcohol and drug addiction. Zuo et al. show that the risk increases in a direct ratio with the number of G alleles at the level of polymorphisms rs6454674 and rs806368. These two polymorphisms frequently associate with drug abuse, when they appear separately, but more intensively when they appear simultaneously [71]. Other studies show the connection between rs1049353 polymorphism and Alcohol withdrawal delirium [72]. Hutchison et al. indicated the existence of an association between the C allele of the rs2023239 polymorphism and alcohol abuse [77].

Ballon et al. show that the frequency of (AAT)12 and (AAT)n polymorphisms is increased in cocaine addicted patients [73]. Nicotine addiction was associated with rs12720071, rs806368, rs2023239 polymorphisms [78].

CNR1 VARIANTS AND AFFECTIVE DISORDERS

Chakrabarti et al. analyze 4 polymorphisms which associate with different striatal responses to happiness but not with disgust [74]. This shows a relationship between CNR1 gene’s variations and social behavior modulation. A study shows that the repetition of (AAT) triplet in CNR1 promotor region cannot be involved in the pathogenesis or in the psychotic symptoms of affective disorders [75]. A study carried out by Ehlers et al. shows the significantly statistical association of impulsivity with several polymorphisms: (AAT)12; (AAT)n/A6; rs1535255, rs2023239, rs1049353 and rs806368 [76]. (Table I).
Table 1

<table>
<thead>
<tr>
<th>Effects on CNS</th>
<th>Polymorphism</th>
<th>Metabolic Effects</th>
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<tbody>
<tr>
<td>Nicotine addiction</td>
<td>rs12720071</td>
<td>↑ Abdominal circumference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Subscapular cutaneous skinfold</td>
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<td></td>
<td></td>
<td>↑ Body mass index</td>
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<tr>
<td>Resistance to antidepressive treatment</td>
<td>rs1049353</td>
<td>↑ Abdominal circumference</td>
</tr>
<tr>
<td>Alcohol withdrawal delirium</td>
<td></td>
<td>↑ Waist to hip ratio</td>
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<tr>
<td>Impulsivity</td>
<td></td>
<td>↑ Body mass index</td>
</tr>
<tr>
<td><em>We found no studies</em></td>
<td>rs806381</td>
<td>↑ Body mass index</td>
</tr>
<tr>
<td><em>We found no studies</em></td>
<td>rs10485170</td>
<td>↑ Body mass index</td>
</tr>
<tr>
<td>Substance addiction (alcohol, drugs)</td>
<td>rs6454674</td>
<td>↑ Body mass index</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>rs806368</td>
<td><em>We found no studies</em></td>
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<tr>
<td>Nicotine addiction</td>
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<tr>
<td>Impulsivity</td>
<td>rs1535255</td>
<td><em>Studies infirmed such correlations</em></td>
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<tr>
<td>Alcohol abuse</td>
<td>rs2023239</td>
<td>↑ Body mass index</td>
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<tr>
<td>Impulsivity</td>
<td></td>
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<tr>
<td>Nicotine addiction</td>
<td>rs806368</td>
<td><em>We found no studies</em></td>
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<tr>
<td>Hebephrenic schizophrenia</td>
<td>(AAT)9</td>
<td><em>We found no studies</em></td>
</tr>
<tr>
<td>Cocaine addiction</td>
<td>(AAT)12</td>
<td><em>We found no studies</em></td>
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<tr>
<td>Impulsivity</td>
<td></td>
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<tr>
<td>Parkinson syndrome</td>
<td>(AAT)n</td>
<td><em>We found no studies</em></td>
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<tr>
<td>Cocaine addiction</td>
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<tr>
<td>Schizophrenia</td>
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**CONCLUSION**

The discovery of ECS and its signaling pathways opens a door towards the understanding of several important physiologic processes regarding appetite, food intake, metabolism, weight gain, motor control, memory, learning, drug addiction and nociception. The detailed analysis and validation of the ECS functioning can bring us very close to the discovery of new diagnosis and treatment methods for obesity, drugs abuse and numerous psychic diseases.
apetitul și balanța energetică, funcționând ca un sistem de recuperare după stres, care rămâne inactiv în condiții fiziologice de repaus. Este implicat în diverse procese fiziologice ca nociceptia, controlul motor, memoria, învățarea, apetitul, ingestia de alimece și balanța energetică. În acest articol se analizează implicarea a 11 polimorfisme ale genei CNR1 în modularea efectelor metabolice și centrale ale SEC. Numeroase studii au arătat că polimorfismele rs12720071, rs104935, rs10485170, rs6454674, rs2023239 sunt asociate cu efe ctele metabolice. Din acestea, polimorfismele rs12720071, rs104935, rs6454674, rs2023239 se asociază și cu efecte centrale ale SEC (dependența de substanțe, impulsivitatea, rezistența la tratamentul antidepresiv). Alte studii au arătat că rs806368, rs1555255, (AAT)9, (AAT)12 și (AAT)n sunt corelate numai cu efe ctele centrale (schizofrenia, dependența de substanțe, impulsivitatea, sindromul Parkinson).

Descoperirea SEC și a căilor sale de semnalizare deschide drumul spre întelegerea mai multor procese fiziologice legate de apetit, aport alimentar, metabolism, creștere ponderală, control motor, memoria, învățarea, dependența de substanțe și nociceptia. Analiza detaliată și validarea funcțiunii SEC ne poate aduce mai aproape de descoperirea unor noi metode de diagnostic și tratament pentru obezitate, abuz de substanțe și numeroase afecțiuni psihiice.

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REFERENCES
1. MECHOLAM R., GAONI Y., A total synthesis of DL-delta-1-tetrahydrocannabinol, the active constituent of hashish. J. Am. Chem. Soc., 1965; 87:3273-3275 [8A].
9. KATONA I., SPERLAGH B., MAGLOCZKY Z., SANTHA E., KOFAVLI A. et al., GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience, 2000; 100: 797–804.
13. KAWAMURA Y., FUKAYA M., MAEJIMA T. et al., The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J. Neurosci., 2006; 26:2991–3001.


82. KOLA B., HUBINA E., TUCCI S.A. et al., Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. J. Biol. Chem. July 1, 2005; 280(26):25196–25201.


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