Brain Cannabinoid CB2 Receptor in Schizophrenia


Background: Neural endocannabinoid function appears to be involved in schizophrenia. Two endocannabinoid receptors, CB1 and CB2, are found in the brain and elsewhere in the body. We investigated roles of CB2 in schizophrenia.

Materials and Methods: An association study was performed between tag single nucleotide polymorphisms (SNPs) in the CNR2 gene encoding the CB2 receptor and schizophrenia in two independent case-control populations. Allelic differences of associated SNPs were analyzed in human postmortem brain tissues and in cultured cells. Prepulse inhibition and locomotor activity in C57BL/6JmmsSlc mice with CB2 receptor antagonist AM630 administration was examined.

Results: The analysis in the first population revealed nominally significant associations between schizophrenia and two SNPs, and the associations were replicated in the second population. The R63 allele of rs2501432 (R63Q) (p = .001), the C allele of rs12744386 (p = .005) and the haplotype of the R63-C allele (p = 5 × 10^-5) were significantly increased among 1920 patients with schizophrenia compared with 1920 control subjects in the combined population. A significantly lower response to CB2 ligands in cultured CHO cells transfected with the R63 allele compared with those with Q63, and significantly lower CB2 receptor mRNA and protein levels found in human brain with the CC and CT genotypes of rs12744386 compared with TT genotype were observed. AM630 exacerbated MK-801- or methamphetamine-induced disturbance of prepulse inhibition and hyperactivity in C57BL/6JmmsSlc mice.

Conclusions: These findings indicate an increased risk of schizophrenia for people with low CB2 receptor function.

Key Words: Association, cAMP, cannabinoid, G protein coupled receptor, gene, methamphetamine, MK-801, mouse, postmortem brain, schizophrenia

Tetrahydrocannabinol (THC) in marijuana acts on cannabinoid receptors, and natural endocannabinoids are important regulators of various aspects of psychobehavioral, immunologic, and metabolic functions. Many relationships between marijuana use and schizophrenia have been reported: a dose-response relationship has been found between the amount of cannabis used in adolescence and the subsequent risk of developing schizophrenia (1,2), more psychotic symptoms are experienced by schizophrenic patients who use cannabis (3), schizophrenia-like symptoms can occur in nonschizophrenic people after cannabis use (4), and there is a decrease in gray matter density in the right posterior cingulate cortex in first-episode schizophrenics who use cannabis compared with those who do not use cannabis (5). Further, biological phenomena related to endogenous cannabinoids such as significantly higher amounts of the endocannabinoid anandamide in the blood occur more frequently in patients with acute schizophrenia than in healthy volunteers (6), and significantly higher levels of anandamide are detected in the cerebrospinal fluid (CSF) of first-episode schizophrenic patients than that of healthy volunteers (7,8).

Endogenous cannabinoids bind to and activate two G protein-coupled receptors, the predominantly central cannabinoid receptor type 1 (CB1) and predominantly peripherally expressing cannabinoid receptor type 2 (CB2). CB1 receptor has mainly been investigated in the endogenous cannabinoid system in the central nervous system (CNS) (9–11). In schizophrenic patients, the cannabinoid CB1 receptor agonist CP-55,940 disrupts sensory gating and neuronal oscillation (12). Some studies have also suggested an association between microsatellite and single nucleotide polymorphism (SNP) genetic markers in the CNR1 gene (encoding the CB1 receptor) and the incidence of schizophrenia (13–16). However, neither nonsynonymous polymorphisms nor polymorphisms in the CNR1 gene that influence the full-length CNR1 transcript expression have been reported.

In addition to human studies, prepulse inhibition (PPI) is frequently used in pharmacobehavioral studies of animal models. PPI inhibition refers to the reduction in amplitude of the

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startle reflex that occurs when a brief, subthreshold stimulus immediately precedes a startle stimulus (17). Deficits in PPI are observed in several psychiatric disorders, particularly in schizophrenia (18), and it has been postulated that this impairment of sensorimotor gating reflects at least some portion of the cognitive dysfunction observed in patients with schizophrenia (19,20). In relation to the cannabinoid system, Long et al. (21) reported that cannabidiol reverses MK-801-induced disruption of PPI in mice.

Recently, attention has been drawn to the expression of the CB2 receptor in the CNS (22–24). This receptor has been reported to be involved in alcohol preference in mice and in human alcoholism (25). Clinical remission of schizophrenia has been reported to be accompanied by significant decreases in anandamide and CNR2 mRNA levels, which encode the CB2 receptor, in peripheral blood mononuclear cells (6). Thus, CB2 receptors may play a role in psychiatric disorders.

In this study, we investigated genetic associations between CNR2 gene polymorphisms and schizophrenia and functions of potentially associated SNPs in cultured cells and human post-mortem brain. The effects of CB2 receptor inverse agonist on mouse behavior were also investigated.

Materials and Methods

Participants in the Association Study

Consensual diagnosis of schizophrenia was made according to the DSM-IV. Control subjects had no history of mental illness, and second-degree relatives were free of psychosis in a brief psychiatric interview. The first screening group of subjects (East Japan) comprised 1152 unrelated Japanese patients with schizophrenia and 1194 control subjects. The second group of subjects (West Japan) comprised 768 unrelated Japanese patients with schizophrenia and 726 control subjects (see details in Methods and Materials section of Supplement 1). HapMap Yoruba subjects were also genotyped because the allele frequency of rs2501432 was not known.

SNP Analysis

Five tag SNPs [rs9424339, rs2502959, rs2501432 (R63Q), rs2229579 (H316T), and rs12744386] were selected using the Haploview version 3.32 software (http://www.broad.mit.edu/mpg/haploview/) from the Japanese subject data in the in the HapMap database (Figure 1). These SNPs contain two common nonsynonymous polymorphisms, and rs12744386, which was associated with different CNR2 gene expression by cis-acting fashion (C allele, effect = −.490, H2 = 11.64, logarithm of odds (LOD) = 8.819) in lymphoblast cells, as listed in the SNP browser 1.01 database (26) (Figure 2A).

Genotypes were determined using TaqMan SNP genotyping assay (Applied Biosystems, Foster City, California) or by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (see details in Methods and Materials of Supplement 1).

Human Postmortem Brains

Brain tissue of gray matter in an area of the prefrontal cortex (Brodman’s area 9 [BA9]) was received from two tissue banks and one research institute (Table S1 and Methods and Materials in Supplement 1).

CNR2 Gene and Protein Expression Analysis

Expression of the CNR2 gene was analyzed using the TaqMan Realtime PCR system with the TaqMan gene expression assays Hs00361490-m1. TaqMan GAPDH Control Reagent and TaqMan β-actin Control Reagents were used to normalize the data, respectively (Applied Biosystems). Expression of the CNR2 protein was analyzed with the Western blot method. Human CB2 Receptor Polyclonal Antibody (1:1000 dilution) (101550, Lot. 165113, Cayman Chemical, Ann Arbor, Michigan) was used as the primary antibody, or polyclonal antibody to beta-Actin (1: 500 dilution; IMG-5142A, Lot. 03,231,897B-04, Imgenex, San Diego, California), for normalization (see details in Methods and Materials of Supplement 1).

Functional Analysis of the CNR2 Gene in Comparison Between R63 and Q63

CB2 receptors with either the CNR2 R63 or Q63 allele were expressed in cultured CHO cells (see details in Methods and Materials of Supplement 1). One millimolar forskolin as final concentration was added to the culture medium for 15 min, and then the endocannabinoid 2-arachidonoylglycerol (2-AG; 1 μmol/L, final concentration; Cayman Chemical), CB2 selective agonist JWH-015 (50 μmol/L, final concentration; Cayman Chemical), or CB2 selective inverse agonist AM630 (100 μmol/L, final concentration) (Tocris Bioscience, Ellisville, Missouri) was administered to the cells. cAMP level was measured 15 min after the administration using the camp-Screen System (Applied Biosystems, Bedford, MA). Effects of those ligands on the cyclic adenosine monophosphate (cAMP) levels were examined in each allele type of CB2 receptor expressed CHO cells (see details in Methods and Materials of Supplement 1). Because the CB2 receptor is a Gi-type G protein-coupled receptor, decreased or increased levels of cAMP were considered the proper response to the agonists or inverse agonists, respectively.

Prepulse Inhibition and Locomotor Tests in Mice

Effects of pretreatment with CB2 ligand AM630 (3 mg/kg or 30 mg/kg intraperitoneally) on drug (either MK-801 or methamphetamine)-induced prepulse inhibition (PPI) and locomotor activity were evaluated in C57BL/6Jmslsc male mice (Japan SLC, Shizuoka, Japan). Experimental procedures are described in Methods and Materials of Supplement 1.

All animal procedures were performed according to protocols approved by the Animal Care and Use Committee of the University of Tsukuba.
Statistics

Deviation from predicted Hardy-Weinberg frequencies, alleric associations, and linkage disequilibrium (LD) between SNPs were evaluated with Haploview software, version 3.11. Because two-stage analyses were performed for genetic associations, SNPs showing nominally significant allelic association ($p < 0.05$) were subsequently analyzed in an independent population. A significant association was defined when the given $p$ value for allelic tests was less than 5% after permutation analysis in the second and combined populations. Haplotype associations were also evaluated by permutation analysis using Haploview software version 3.11. The global haplotype association was tested with COCAPHASE from the UNPHASED software 2.403 (27).

The relationship between $CNR2$ gene expression levels and diagnosis of schizophrenia was analyzed by $t$ test. Relationships between $CNR2$ gene expression or protein expression levels and $rs12744386$ genotypes were analyzed using one-way analysis of variance (ANOVA), followed by post hoc analysis using a Student's $t$ test. The correlation between $CNR2$ expression levels and either postmortem interval (PMI) or pH in brain tissues was assessed by linear regression analysis. The relationship between $CNR2$ gene expression and diagnosis of schizophrenia was analyzed by $t$ test to evaluate effect of AM630 pretreatments with two doses on either MK-801 or methamphetamine induced PPI, respectively (Bonferroni correction for two comparisons). The effect was then analyzed at each prepulse intensity. Effects of drug treatment, AM630 pretreatments, and the interaction on locomotor activity were analyzed by ANOVA, followed by post hoc analysis using a Student’s $t$ test to evaluate effect of two doses of AM630 pretreatments (Bonferroni correction for two comparisons). ANOVA and Student’s $t$ tests were carried out using JMP software version 5.1 (SAS Institute, Japan).

Results

Table 1 shows the distribution of the genotypes of the tag SNPs in the $CNR2$ gene region, and Figure 1 shows the LD pattern within those tag SNPs. None of the SNPs deviated significantly from the expected Hardy-Weinberg equilibrium. Nominally significant differences were found in allele frequencies of $rs12744386$ ($p = 0.05$, odds ratio [OR] = 1.13 with 95% confidence interval [CI]: 1.00–1.27) and $rs2501432$ (R63Q) ($p = 0.003$, OR = 1.19, 95% CI: 1.02–1.37) between schizophrenic and control subjects from east Japan (Table 1). The significant differences were replicated in subjects from west Japan; $rs12744386$ (OR = 1.19, 95% CI: 1.03–1.38, permutation $p = 0.04$) and $rs2501432$ (R63Q) (OR = 1.19, 95% CI: 1.02–1.37, permutation $p = 0.04$) between schizophrenic and control subjects from west Japan (Table 1).

In the combined populations, the distributions of allele frequencies between schizophrenic and control subjects were...
Table 1. Genotype and Allele Distributions of tag Single Nucleotide Polymorphisms (SNPs) in the CNR2 in the Case Control Subjects

<table>
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<th>SNP</th>
<th>Samples (n)</th>
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<th>p</th>
<th>Allele Count (Frequency)</th>
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<td></td>
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Replication Study

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<td>37%</td>
<td>852</td>
<td>46%</td>
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significantly different for rs12744386 (OR = 1.15, 95% CI: 1.05–1.26, permutation p = .005) and rs2501432 (R63Q) (OR = 1.19, 95% CI: 1.09–1.31, permutation p = .001) (Table 1). Because of the relatively strong LD between rs12744386 and rs2501432 ($r^2 = .67$), the haplotype was constructed with these two SNPs to examine the association between the haplotype and schizophrenia. As a result, the haplotype of the C allele of rs12744386 and R63 allele showed a strong association with schizophrenia in our Japanese subjects (OR = 1.26, 95% CI: 1.15–1.37, permutation $P = 5 \times 10^{-10}$), which was also observed independently in two groups of subjects (Table 2). The global haplotype association value was 5.1 $\times 10^{-10}$.

rs12744386 was suggested to be associated with CNR2 mRNA levels in lymphoblastoid cell lines by SNP Browser (allele C: Effect = −.49, H2 = 11.64, LOD = 8.819; Figure 2A). This association was confirmed in postmortem prefrontal brain samples. The rs12744386 genotypes were associated with the CNR2 mRNA levels in the brain [GAPDH-normalized, F(2,45) = 6.7, $p = .003$; ACTB-normalized, F(2,45) = 4.9, $p = .01$; Figure 2B], and with the CNR2 protein levels in the brain [F(2,38) = 6.3, $p = .005$; Figure 2C] with the lowest expression in the CC genotype and the highest expression in the TT genotype. Post hoc analysis showed a significant difference between TT and other genotypes (Figure 2B). There was a significant correlation between results for gene expression normalized by two internal controls, GAPDH and ACTB ($r^2 = .67$, $F(1,45) = 87.83$, $p < .0001$). There was also significant correlation between results for gene expression normalized by ACTB and protein expression normalized by ACTB ($r^2 = .12$, $F(1,33) = 4.2$, $p = .05$). There were no significant correlations between PMI and CNR2 expression [GAPDH-normalized, $F(1,44) = .6$, NS; β-actin-normalized, $F(1,44) = .7$, ns; pH and CNR2 expression [GAPDH-normalized, $F(1,43) = .4$, NS, ACTB-normalized, F(1,43) = .05, ns; or diagnosis of schizophrenia and CNR2 expression [GAPDH-normalized, $F(1,45) = .4$, ns, ACTB-normalized, F(1,45) = 1.4, NS].

Regarding the rs2501432 (R63Q) polymorphism, residue 63 of the CB2 receptor protein of the mouse (Mus musculus), rat (Rattus norvegicus), rhesus monkey (macaca mulatta), and chimpanzee (Pan troglodytes) is arginine (R63), which is also the major allele of humans (Figure S1 in Supplement 1). A similar allelic frequency for Q63 (of rs2502959) was observed both in Japanese and Caucasian populations (approximately 45%). A...
lower frequency was observed in an African population (17.3%; Figure S1 in Supplement 1). Analysis using the GENETYX software (Genetyx Corporation, Tokyo, Japan; http://www.sdc.co.jp/genetyx/) predicted a structural change of the receptor by the amino acid substitution and by Chou-Fasman, Robson, or hydrophilic/hydrophobic structure analysis (Figure S2 in Supplement 1).

In this study, responses of relative cAMP levels to saline and 2-AG, AM630, or JWH-015 in Q63- or R63-allele CNR2-transfected CHO cells were measured. CB2 receptor ligands did not change cAMP levels in the untransfected CHO cells. ANOVA revealed significant main effects of allele [F(1,17) = 7.5, p = .01] and of the interaction between 2-AG and allele [F(1,17) = 7.5, p = .01], but not of 2-AG [F(1,17) = .4, ns]. Further, ANOVA revealed significant main effects of AM630 administration [F(1,29) = 14.8, p = .0006], of allele [F(1,29) = 4.2, p = .05], and of the interaction between AM630 administration and allele [F(1,29) = 4.2, p = .05]. ANOVA showed trends of main effects of allele [F(1,61) = 3.0, p = .09, JWH-015 administration [F(1,61) = 3.1, p = .08], and the interaction between JWH-015 administration and allele [F(1,61) = 3.0, p = .09]. Post hoc analysis demonstrated that relative cAMP levels were reduced in CHO cells with the Q63 type of CB2 receptor expressed, in response to both agonists, 2-AG (corrected p = .09) and JWH-015 (corrected p = .03). No significant reduction of cAMP levels was observed in those with the R63 type of CB2 receptor in response to both ligands (Figure 3A,B). Levels of cAMP in CHO cells with the Q63 type of CB2 receptor were significantly less than in those with the R63 type CB2, in response to both agonists, 2-AG (corrected p = .002) and JWH-015 (corrected p = .05), respectively. The inverse agonist AM630 increased cAMP levels significantly more in CHO cells with the Q63 allelic CB2 receptor (corrected p = .01), but it was not significant in those with the R63 allelic CB2 receptor. Levels of cAMP were increased more significantly in CHO cells with the Q63 than in those with the R63 allelic CB2 receptor (corrected p = .06; Figure 3C). Unless the CB2 receptor was expressed in CHO cells, cAMP levels did not change in response to JWH-015 and to AM630 (Figure 3B and 3C).

We evaluated the effect of pretreatment with the CB2 receptor inverse agonist AM630 on PPI, combined with MK-801 or methamphetamine treatment separately, in mice. Alone, the CB2 receptor inverse agonist AM630 did not affect PPI in mice. ANOVA revealed significant main effects of prepulse intensity [F(3,303) = 42.4, p < .0001] and of methamphetamine [F(4,304) = 25.5, p < .0001], but not of the interaction [F(12,312) = 1.4, ns]. Post hoc analysis revealed pretreatment with AM630 at a dose of 30 mg/kg produced a significantly greater reduction in % PPI than MK-801 treatment alone (p = .002), but pretreatment with AM630 at the dose of 3 mg/kg did not significantly reduce % PPI (p = .2) compared with saline pretreatment. When analyzed at each prepulse intensity, the reduction of % PPI by pretreatment with AM630 at the dose of 30 mg/kg was observed at 85 dB prepulse intensity (p = .05; Figure 4A).

ANOVA revealed significant main effects of prepulse intensity [F(3,207) = 38.1, p < .0001] and of methamphetamine [F(4,208) = 3.3, p = .01], but not of the interaction [F(12,216) = .3, ns]. Post hoc analysis revealed pretreatment with AM630 at a dose of 3 mg/kg produced a significantly greater reduction in % PPI than methamphetamine treatment alone (p = .04), and pretreatment with AM630 at a dose of 30 mg/kg produced a significantly greater reduction in % PPI than methamphetamine treatment.
alone (\(p = .03\)). Although the overall effect was significant, post hoc tests did not reveal significant differences at individual prepulse intensities (Figure 5A).

ANOVA revealed significant effects of either MK-801 or methamphetamine administration on the locomotor activity of mice in home cages \(F(5,399) = 69.2, p < .0001, F(5,339) = 94.2, p < .001\), respectively, Figures 4B and 5B). Both drugs produce significant hyperlocomotion. Post hoc analysis revealed that although AM630 alone did not produce significant hyperlocomotion, AM630 pretreatments at doses of both 3 mg/kg and 30 mg/kg significantly increased methamphetamine-induced locomotion (both \(p < .0001\)). Also, AM630 pretreatments at a dose of 30 mg/kg significantly increased MK-801-induced locomotion \((p < .0001)\) than saline pretreatments.

![Figure 3.](image3.png) Functional analysis of the cannabinoid CB2 receptor with R63Q polymorphism. In cultured CHO cells expressed cloned human CB2 receptor, forskolin-evoked cyclic adenosine monophosphate (cAMP) level was regulated by three kinds of CB2 receptor ligands in this cell signaling system. CB2 receptor function in response to the ligands between Glu63 and Arg63 in the CNR2 was analyzed. Black bars present cAMP levels in saline-treated CHO cells, and white bars show those in CHO cells treated with the ligand. Nominal \(p\) values are shown. (A) When endocannabinoid 2-AG binds to CB2 receptor, the evoked cAMP level is downregulated in CHO cells with Glu63 type CB2 receptor but not regulated in those with Arg63 type receptor. (B) When CB2 selective agonist JWH-015 binds to CB2 receptor, the evoked cAMP level is downregulated in CHO cells with Glu63 type CB2 receptor but not regulated in those with Arg63 type receptor or in naive CHO cells. (C) When CB2 selective inverse agonist AM630 binds to CB2 receptor, the evoked cAMP level is upregulated in CHO cells with Glu63 type CB2 receptor but not regulated in those with Arg63 type receptor or in naive CHO cells.

![Figure 4.](image4.png) MK-801 induced alteration of prepulse inhibition (PPI) and locomotion influenced by AM630. (A) The vertical axis shows % PPI. Saline pretreated and treated before PPI test (Saline/Saline, \(n = 14\), AM630 (3 mg/kg intraperitoneal [IP]) pretreated and saline treated before PPI test (AM630 (3 mg/kg)/ saline, \(n = 6\)), saline pretreated and MK-801 (5 mg/kg IP) treated before PPI test (Saline/MK-801, \(n = 24\)), AM630 (3 mg/kg IP) pretreated and MK-801 (5 mg/kg IP) treated before PPI test (AM630 (3 mg/kg)/MK-801, \(n = 27\)), AM630 (30 mg/kg IP) pretreated and MK-801 (5 mg/kg IP) treated before the PPI test (AM630 (30 mg/kg)/MK-801, \(n = 9\)). Nominal \(p < .05\) is shown. (B) The vertical axis shows locomotion activity in the home cage for every 20-min time period. The horizontal axis shows as follows: pre, 10 min after pretreatment and 20 min before treatment; 20, 40, 60, 80, 100, 120, 140, 160, and 180, minutes from MK-801 injection (5 mg/kg IP) 30 min after pretreatment. Saline pretreated and treated (Saline/Saline, \(n = 8\)), 3 mg/kg AM630 pretreated and saline treated (AM630 (3 mg/kg)/saline, \(n = 6\)), 30 mg/kg AM630 pretreated and saline treated (AM630 (30 mg/kg)/saline, \(n = 7\)), saline pretreated and MK-801 treated (saline/MK-801, \(n = 8\)), 3 mg/kg (AM630 pretreated and MK-801 treated AM630 (3 mg/kg)/MK-801, \(n = 8\)), 30 mg/kg AM630 pretreated and MK-801 treated (AM630 (30 mg/kg)/MK-801, \(n = 9\)), saline pretreated and MK-801 treated (saline/MK-801, \(n = 8\)), 3 mg/kg AM630 pretreated and MK-801 treated (AM630 (3 mg/kg)/MK-801, \(n = 5\)), saline pretreated and MK-801 treated (saline/MK-801, \(n = 5\)), and 30 mg/kg AM630 pretreated and MK-801 treated (AM630 (30 mg/kg)/MK-801, \(n = 5\)). *Nominal \(p < .05\) is shown for difference of activity between saline/MK-801 and either AM630 (3 mg/kg)/MK-801 in dark gray or AM630 (30 mg/kg)/MK-801 in black at each time period.
Figure 5. Methamphetamine-induced alteration of prepulse inhibition (PPI) and locomotion influenced by AM630. (A) The vertical axis shows % PPI. Saline pretreated and treated before PPI test (Saline/Saline, n = 10); AM630 (3 mg/kg intraperitoneal [IP]) pretreated and saline treated before PPI test (AM630 [3 mg/kg]/saline, n = 6); saline pretreated and methamphetamine treated (2 mg/kg IP) before PPI test (saline/methamphetamine, n = 16); AM630 (3 mg/kg IP) pretreated and methamphetamine treated before PPI test (AM630 [3 mg/kg]/methamphetamine, n = 17); AM630 (30 mg/kg IP) pretreated and methamphetamine treated before PPI test (AM630 [30 mg/kg]/methamphetamine, n = 7). (B) The vertical axis shows locomotion activity in the home cage for every 20 min. The horizontal axis shows as follows: pre, 10 min after pretreatment and 20 min before treatment; 20, 40, 60, 80, 100, 120, 140, 160, and 180, minutes from methamphetamine injection (2.0 mg/kg IP) 30 min after pretreatment. Saline pretreated and treated (Saline/Saline, n = 8), 3 mg/kg AM630 pretreated and saline treated (AM630 [3 mg/kg]/saline, n = 6), 30 mg/kg AM630 pretreated and saline treated (AM630 [30 mg/kg]/saline, n = 7), saline pretreated and methamphetamine treated (saline/methamphetamine, n = 5), 3 mg/kg AM630 pretreated and methamphetamine treated (AM630 [3 mg/kg/methamphetamine, n = 5], 30 mg/kg AM630 pretreated and methamphetamine treated (AM630 [30 mg/kg/methamphetamine, n = 5]. *Nominal p < .05 is shown for difference of activity between saline/methamphetamine and either AM630 (3 mg/kg/methamphetamine in dark gray or AM630 (30 mg/kg/methamphetamine in black at each time period.

Discussion

We found two SNPs in and near the CNR2 gene associated with schizophrenia in Japanese populations. One of these two SNPs, rs12744386, was found to be associated with gene expression levels (the risk allele was associated with low levels of CNR2) and another, rs2501432, was a missense R63Q. By the transfection experiments, we showed that, compared with the protective allele, the risk allele of the missense polymorphism had a poor response to CB2 ligands. Therefore, it was thought that both risk alleles of the two SNPs associated with schizophrenia were related to the direction of lower functioning of CNR2. Furthermore, these two SNPs were in LD with each other (r² = .67), and a haplotype composed of these two alleles associated with a lower functioning of CNR2 had a greater association with schizophrenia than with the allelic association of either SNP alone. These findings provide evidence for the association between the CNR2 gene and schizophrenia, although, because of the haplotype structure in the Japanese population, there is a possibility that the strength of association of either SNP might be inflated by the other SNP.

This study confirms that rs2501432 (R63Q) of CNR2 gene is functional. The CB2 receptor is a guanine nucleotide–binding protein (G protein) coupled receptor, which is a member of the Gi-type receptor family. Codon 63 is located at the first intracellular domain immediately after the first transmembrane domain. Alteration of hydrophilic/hydrophobic structure at this point may alter signal transduction efficiency in cells after ligand stimulation. Our finding of a less efficient response to endocannabinoid 2-AG in the common R63 type than in the minor Q63 type is consistent with the findings of endocannabinoid-induced inhibition of lymphocyte proliferation in human peripheral tissue (25,28,29). Our study identifies that rs12744386 is a marker associated with changes in CNR2 mRNA expression (in cis-acting fashion) both in lymphoblasts and brain tissue. Because the haplotype of the R63 and the C allele was more significantly associated with schizophrenia than either allele alone, it is possible that genetic variations in the CNR2 genome region related to the reduced function of CB2 receptors may synergistically confer susceptibility to schizophrenia.

The genetic findings of lower functioning of CNR2 associated with schizophrenia may be supported by the findings of the pharmacologic experiments using the animal model and CB2 antagonists in this study. MK-801- or methamphetamine-induced disturbance of PPI has been used as an animal model of schizophrenia (30–32). MK-801 reduced PPI, and when MK-801 was used in combination with the CB2 inverse-agonist AM630, PPI was further reduced. However, AM630 alone did not have any effect on PPI in any prepulse-pulse sessions. Methamphetamine alone at the dose of 2.0 mg/kg did not show a significant change in PPI; however, when methamphetamine was used in combination with AM630, PPI was significantly reduced. AM630 also enhanced MK-801- and methamphetamine-induced hyperlocomotion in mice, although there was no effect of AM630 alone. Therefore, reduced CB2 functioning itself is not likely to cause schizophrenia, but it is hypothesized that, when combined with other risk factors, it could be harmful for schizophrenia-susceptible individuals. Furthermore, because these pharmacologic models are related with the glutamatergic and dopaminergic neural systems underlying schizophrenia, our study indicates that CB2 function is related to these neural systems.

CB2 receptors perform many functions. An animal model for the neurodevelopmental hypothesis of schizophrenia was demonstrated using neonatal lesions in hippocampus, when pregnant mother rats were exposed to the bacterial endotoxin lipopolysaccharide (33,34). Although the CB2 receptor has been known to be linked to the immune system in the peripheral
body, its function was also involved in neural progenitor proliferation in the hippocampus (35,36). De la Fuente et al. (37) proposed an effect of maternal deprivation in neuroimmunoendocrine interactions. Maternal deprivation has also been shown to lead to a disruption of PPI and startle habituation, as well as reduced latent inhibition (38–41). The effect of amphetamine was enhanced in early maternally deprived rats (42). At the same time, early maternal deprivation induced an increase in the number of degenerating hippocampal neurons and astrocytes and increased corticosterone and 2-AHP hippocampal levels (43,44). Therefore, this animal model of a specific aspect of schizophrenia also has implications on the endocannabinoid system. Another recent study showed an analysis of the effects of early maternal deprivation on CB1 and CB2 receptors in the hippocampus, indicating a decrease of CB1 receptor expression and an increase of CB2 receptor expression (45). The present study provided further evidence for the involvement of CB2 in schizophrenia.

The present study had some limitations. The possible effect of AM630 on CB1 and interaction between CB1 and CB2 receptors could not be excluded. A further study using Cnr1 knockout mice is needed to explore this pharmacologic possibility and clarify the functions of Cnr2 in brain. Many genetic association studies have not been replicated and confirmed in other populations, particularly in the case of psychiatric disease. Replication studies in other ethnic populations are needed to confirm whether the CB2 receptor plays a role in susceptibility to schizophrenia. Several genomewide Association studies (GWAS) that have been reported or are being conducted may enable us to compare the allele frequencies of many SNPs in other populations. However, GWAS data sets with Affymetrix 500K or Illumina HumanHap550 platforms did not include the SNPs of rs12744386 or rs2501432, which were found to be associated with schizophrenia in this study. Particularly, the allele frequencies of rs2501432 are not recorded in the DS SNP database.

In conclusion, this study indicates that a genetically predetermined lower functioning of CB2 receptors increases susceptibility to schizophrenia when combined with other risk factors. However, this simple understanding needs to be further elucidated because CB2 receptors perform many functions, and the incidence of the R63 allele is found more commonly in patients who have other psychiatric or physiologic disorders, such as alcoholism, major depression, autoimmune disease, or osteoporosis (25,28,29).

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