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Novel G423S Mutation of Human α7 Nicotinic Receptor Promotes Agonist-Induced Desensitization by a Protein Kinase C-Dependent Mechanism

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ABSTRACT

The α7 nicotinic acetylcholine receptor subunit (*CHRNA7*) gene harbors a high degree of polymorphism. In this study, we found a novel variant (1267 G to A) in exon 10 of the *CHRNA7* gene in a Japanese population. This variant results in glycine-to-serine substitution at position 423 (G423S) located in the large cytoplasmic loop of the protein. To clarify the possibility that the G423S mutation alters the pharmacological properties of α7 receptors, acetylcholine (ACh)-elicited current through α7-G423S mutant receptors expressed in *Xenopus laevis* oocytes was measured using the two-electrode voltage-clamp technique. We found that the current elicited by ACh (1 mM, 5 s) through α7-G423S receptors, but not through α7 receptors, was significantly decreased by treatment with a protein kinase C activator, phorbol-12-myristate-13-acetate (PMA, 10–30 nM). In addition, PMA (10 nM) selectively promoted a progressive decrease in α7-G423S current induced by repetitive application of ACh pulses (1 mM, 0.1 s, 0.17–0.33 Hz) compared with α7 current. PMA also enhanced the inactivation of α7-G423S mutant receptors induced by a prolonged application of choline (30 μM) without affecting α7 receptor responses. Western blot analysis showed that the treatment with PMA (30 nM) increased the serine phosphorylation level of the α7-G423S mutant receptors but not that of the wild-type receptors. These findings demonstrate that the G423S mutation promotes receptor desensitization by a protein kinase C-dependent mechanism. Thus, we provide the first evidence that a variant in the human *CHRNA7* gene alters the function of α7 nicotinic receptors.

Neuronal nicotinic acetylcholine receptors are ligand-gated ion channels that are widely distributed in the mammalian brain (Changeux et al., 1998; Paterson and Nordberg, 2000; Gotti and Clementi, 2004). The α7 subunit-containing receptor is one of the predominant subtypes of brain nicotinic receptors, although its distribution in the human brain has not been characterized completely. Based on the finding that α7 receptors are more diffusely distributed in the monkey brain than in the rodent brain, α7 receptors seem to play important roles in primate brain functions (Paterson and Nordberg, 2000; Gotti and Clementi, 2004). The highest expression level is detected in regions that are involved in the processing of sensory information, such as the hippocampus and thalamus (reticular, geniculate) (Gotti and Clementi, 2004). In particular, the α7 receptor-mediated release of GABA from hippocampal interneurons is responsible for the filtering or gating of auditory information passing through the hippocampus (Leonard et al., 1996).

Sensory gating deficit expressed by a poor inhibition of the P50 auditory event-evoked response is an early clinical symptom of schizophrenia and is characterized by a decrease in normal brain ability to inhibit responses to unimportant stimuli (Freedman et al., 2003). This failure of inhibition is associated with poorly sustained attention (Martin et al., 2004). A series of clinical and pathophysiological studies have indicated that reduction in α7 nicotinic receptor expression and function is involved in the sensory gating deficit (Freedman et al., 2000; Weiland et al., 2000). In addition,

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ABBREVIATIONS: SNP, single nucleotide polymorphism; ACh, acetylcholine; DMSO, dimethyl sulfoxide; EGFP, enhanced green fluorescence protein; MLA, methyllycaconitine; NMDA, *N*-methyl-D-aspartate; ORF, open reading frame; PMA, phorbol-12-myristate-13-acetate; PVDF, polyvinylidene fluoride; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; bp, base pair(s).

impaired auditory sensory gating has been linked to the human α7 nicotinic receptor subunit (*CHRNA7*) gene on the chromosome 15q13-q14 locus (Freedman et al., 1997), and many studies have linked schizophrenia to a locus at or near the *CHRNA7* gene (Martin et al., 2004). Leonard et al. (2002) have searched for single-nucleotide polymorphisms (SNPs) in the *CHRNA7* gene promoter region and found that several SNPs are more prevalent in patients with schizophrenia than in control subjects. Furthermore, these SNPs are associated with both decreased promoter activity in vitro and failure to inhibit the P50 auditory-evoked response in humans. This finding, however, is not necessarily consistent with studies of other populations (Houy et al., 2004; Li et al., 2004).

Influence of single amino acid substitution in the α7 nicotinic receptors has been intensively studied using site-directed mutagenesis (Placzek et al., 2005; McLaughlin et al., 2006). Yet, little is known about the polymorphisms located in the coding region of the human *CHRNA7* gene, because the existence of a duplicated α7 receptor subunit (*CHRFAM7A*) gene, in which exons 5 to 10 overlap with those in the *CHRNA7* gene (Gault et al., 1998), complicates polymorphism screening. Gault et al. (2003) have identified three nonsynonymous variants in the human *CHRNA7* gene by a reverse transcriptase-polymerase chain reaction (PCR)-based strategy for variant mapping. However, the impact of the amino acid substitution by these variants on receptor function has not been studied. Thus, the significance of variants in the human *CHRNA7* gene has long been unclarified.

In this study, we analyzed the *CHRNA7* gene in a Japanese population and found a novel SNP that causes a single amino acid substitution (G423S mutation) in the α7 receptor subunit. Furthermore, we investigated the pharmacological and biochemical properties of the mutant receptors ectopically expressed in *Xenopus laevis* oocytes. To our knowledge, this is the first demonstration that a variant in the human *CHRNA7* gene alters the function of the α7 nicotinic receptors.

Materials and Methods

Subjects. We recruited 100 unrelated Japanese subjects with schizophrenia that was diagnosed on the basis of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and 100 Japanese control subjects at Toyama University Hospital and Hokuriku National Hospital (Toyama, Japan). The patient group comprised 58 men and 42 women (mean age ± S.D., 35 ± 11 years), whereas the control group comprised 46 men and 54 women (mean age ± S.D., 32 ± 15 years). The mean total score of the Brief Psychiatric Rating Scale, an estimated rating of psychiatric symptoms, of patients with schizophrenia included in this study was 41 ± 1. Before collecting blood samples, the purpose and risks of this study were precisely explained both orally and in writing, and written informed consent was obtained from all the subjects. The protocol of this study is in accordance with the Declaration of Helsinki and was approved by the ethics committees of the University of Toyama and Hokuriku National Hospital.

Materials. Calphostin C, methyllycaconitine (MLA), phorbol-12-myristate-13-acetate (PMA), and 4α-PMA were purchased from Sigma (St. Louis, MO). Calphostin C, PMA, and 4α-PMA were dissolved in dimethyl sulfoxide (DMSO; Sigma) and diluted in low-Ca^{2+} Ringer's solution (see below). Other standard reagents were purchased from Sigma or Wako Pure Chemicals Industries (Osaka, Japan).

Genetic Analysis. Blood samples were obtained from all the subjects, and genomic DNA was extracted from peripheral leukocytes using a Wizard Genomic DNA purification kit (Promega, Madison, WI). PCR-single-strand conformation polymorphism (SSCP) analysis and sequencing were performed to identify SNPs in the *CHRNA7* and *CHRFAM7A* genes as described previously (Ishihara et al., 2003). Table 1 shows biotinylated primers used for amplification of the 5′-untranslated region, exons, and exon-intron boundaries of the *CHRNA7* gene and for amplification of gene sequences encoding exons 5 to 10, in which 5′-end sequences are specific for either the *CHRNA7* or *CHRFAM7A* gene. The primers were designed on the basis of sequences available in GenBank (accession number NT_010194). For the amplification of exons 1, 2, 3, and 4 of the *CHRNA7* gene, PCR was carried out using a KOD-Plus DNA polymerase kit (Toyobo, Osaka, Japan) under the following conditions: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 98°C for 10 s, and annealing and elongation at 68°C for 20 to 40 s. For the amplification of exons 5 to 10 of the *CHRNA7* or *CHRFAM7A* gene, primary long-range PCR was performed using an LA *Taq* DNA polymerase kit (Takara Bio, Shiga, Japan) and specific primers under the following conditions: initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 98°C for 20 s, annealing at 68°C for 18 min, and final elongation at 68°C for 20 min. The second PCR was performed using the diluted primary PCR product as the template and primers to amplify each exon. The PCR conditions were the same as those used to amplify exons 1 to 4. Thereafter, PCR products were subjected to SSCP analysis. The samples were heated to 95°C for 5 min and immediately cooled on ice until loading. Single-stranded fragments were separated by electrophoresis on Mutation Detection Enhancement gel (FMC Bioproducts, Rockland, ME) at 4°C and detected with an Imaging High-Chemilumi kit (Toyobo) according to the manufacturer's instructions. All SSCP variants were directly sequenced using a BigDye terminator kit and an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA).

Plasmid Construction. The α7 open reading frame (ORF) fragment was amplified from the human brain cDNA library (human brain, cerebellum quick-clone cDNA; Clontech, Mountain View, CA) by PCR using a KOD-plus DNA polymerase kit with the following primers: the sense primer containing a BamHI restriction site (5′-CGCGGATCCCGCTGCAGCTCCGGGACTCAACATG-3′) and the antisense primer containing an AgeI restriction site (5′-GGACCG-GTCGCAAAGTCTTTGGACACGGCCTCCA-3′). The primers were designed on the basis of the sequence available in GenBank (accession number NM_000746). The α7 ORF fragment was cloned into the pGEM-T Easy vector (Promega) and then subcloned into the BamHI and NotI restriction sites of the pcDNA3.1+ vector (Invitrogen, Carlsbad, CA). The original stop codon was generated by site-directed mutagenesis using the KOD-plus DNA polymerase kit with the following paired primers: 5′-CCGTGTCCAAAGACTTTGCGTA-AGGTCCAATCGAATTCCCGCGG-3′, and 5′-CCGCGGGAATTCG-ATTGGACCTTACGCAAAGTCTTTGGACACGG-3′. α7-G423S cDNA was generated by site-directed mutagenesis with the paired primers 5′-GCACCTCCTGCACAGCGGGCAACCCCCCG-3′ and 5′-CGGGGGGTTGCCCGCTGTGCAGGAGGTGC-3′ on the α7 pcDNA-3.1+ vector. The expression vector of the enhanced green fluorescence protein (EGFP)-α7 subunit chimera was constructed by inserting the α7 ORF fragment into the BamHI and AgeI restriction sites of the pEGFP-N1 vector (Clontech). To avoid the interference between the α7 receptor and the EGFP protein, nucleotides encoding a linker consisting of four glycine residues were further inserted between the nucleotide sequences of α7 ORF and EGFP by site-directed mutagenesis with the following paired primers: 5′-GGAGGAGGAG-GAACGGTCGCCACCATGGTGAGCAAGGG-3′ and 5′-CGCAAAGT-CTTTGGACACGGCCTCCACGAAG-3′. The nucleotide sequences of the inserts were confirmed by sequencing.

Expression in *X. laevis* Oocytes. *X. laevis* oocytes expressing recombinant nicotinic receptors were prepared as described previously (Tsuneki et al., 2004). In brief, oocytes were surgically removed

from *X. laevis* frogs under anesthesia and enzymatically isolated. Stage V to VI oocytes were selected and nanoinjected with 23 ng of cDNAs to the nucleus. Oocytes were incubated at 19°C in 100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvic acid, 1% bovine serum albumin, and 25 μg/ml gentamicin, pH 7.5, for 3 to 7 days before electrophysiological recording was carried out. Animal experiments were performed in accordance with the guidelines approved by the University of Toyama Animal Research Committee.

Electrophysiological Recording. α7 Nicotinic current evoked by acetylcholine (ACh) or choline was recorded as described previously (Tsuneki et al., 2004). An oocyte was placed in a tube-like chamber in which low-Ca^{2+} Ringer's solution (82.5 mM NaCl, 2.5 mM KCl, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.4) was perfused by gravity (15 ml/min) to minimize the activation of endogenous Ca^{2+}-activated chloride channels in the oocytes. Atropine (1 μM) was also present in the solution to block endogenous muscarinic receptors. Current was recorded under a two-electrode voltage clamp at a holding potential of −60 mV using a GeneClamp 500 amplifier and pCLAMP7 software (Axon Instruments, Sunnyvale, CA). The sampling rate was 20 Hz. Electrodes contained 3 M KCl and had resistances of <2 MΩ. To apply the nicotinic agonist to the oocyte, the perfusion fluid was switched to a fluid containing the agonist, using a three-way Teflon solenoid valve (Parker Hannifin Corporation, General Valve, NJ) controlled by a PC computer with pCLAMP7 software. The duration of agonist application was 5 s, excluding short pulses (0.1 or 0.2 s) of ACh as described in the legends to Figs. 4 and 5. In the case of a 5-s application of the nicotinic agonist, a 3-min wash produced reproducible control current with no obvious desensitization. To avoid artifacts due to capacitive charging of the oocyte membrane, the current elicited every 3 min by the agonist was recorded until it became stable. A selective antagonist of the α7 receptors, MLA, was applied 6 min before measuring the current elicited by ACh coapplied with MLA. In

TABLE 1
PCR primers for amplification of the human *CHRNA7* gene

	Primer Sequence	PCR Product Size
		bp
Promoter		
Sense	5′-CAGAATTGTCCCGGCTTTCTCCCG-3′	320
Antisense	5′-GCCTCTCCACGTGACGAGCCCC-3′	
Promoter + exon 1		
Sense	5′-GTTCCCTGGGTGGCCGCCGAGA-3′	335
Antisense	5′-TCCCACGGAGGAGTGGAGGGCG-3′	
Exon 2		
Sense	5′-CCCCCTGCCCGGGTCTTCTCTCCTTA-3′	237
Antisense	5′-ACGCTGTCTAAAAAGCCCCTCGGAGC-3′	
Exon 3		
Sense	5′-CACACACAACAACGCTCTCGACAG-3′	207
Antisense	5′-TCCTAAACAGGACCTCTCAGAAGCAA-3′	
Exon 4		
Sense	5′-CAAGGAAGTGAAGTGCTGCTAATGTC-3′	211
Antisense	5′-ACGTGATAGCTACATGTATGCAAGCA-3′	
Exon 5 to exon 10[a]		
Sense	5′-CTGCAGTTCAGTCATTCAAGATATATATGTGGG-3′	17,367
Antisense	5′-CACTTCTACTTGTTTCTAAAGACACTGTAACAG-3′	
Exon 5 to exon 10[a]		
Sense	5′-CCACTTCCAGTGAGGTTTTCTCTGAC-3′	16,480
Antisense	5′-CAGGCAGTGGGCTAATGGGCAAAAT-3′	
Exon 5		
Sense	5′-GGTCTTTGCTGCTCCATCAAAGACAG-3′	238
Antisense	5′-ACTGACTGACACCCAAACTCGCTTCA-3′	
Exon 6		
Sense	5′-CAGCACATCTCAGTCAGCTTCCGTTT-3′	265
Antisense	5′-GAATAGGAAAGCTTTCTTCCAGGCGG-3′	
Exon 7		
Sense	5′-AACAATGGCTCCTTCTCTCCTCCTCC-3′	279
Antisense	5′-CAGCAGAAGGTCTCCAGTCTCAGACT-3′	
Exon 8		
Sense	5′-AGAGAGCCCTCGTTAGACAGAATTGA-3′	226
Antisense	5′-AAATCCTGGGCACACTCTAACCCTAA-3′	
Exon 9		
Sense	5′-CAGAGAACCTGATCAGGGTGTGCCT-3′	355
Antisense	5′-TGGGTGACATAGTGAGACTCCGTCT-3′	
Exon 10–1		
Sense	5′-CCGCCTCAGGGCTGCTCTTAACG-3′	316
Antisense	5′-GTGCAGGAGGTGCTCATCGTGCG-3′	
Exon 10–2		
Sense	5′-GACTCTGGGGTAGTGTGTGGCCG-3′	365
Antisense	5′-CGCCAAGCCAAAGGCCTTGCCCA-3′	
Exon 10–3		
Sense	5′-GGCCGTGTCCAAAGACTTTGCGTAA-3′	306
Antisense	5′-CAGGCAGTGGGCTAATGGGCAAAAT-3′	
Exon 10–4		
Sense	5′-TATCCTTGGCACATCCGTACCAT-3′	353
Antisense	5′-CATCAAGCTGTTTCTCTCTACCGTC-3′	
Exon 10–5		
Sense	5′-CTCTTAGCTTTTCTGCAATTCAAC-3′	357
Antisense	5′-TGGGCTGTTAATTCTAGTAGAACTC-3′	

[a] Primers used in primary PCR.

experiments examining the effects of PMA and 4α-PMA, the vehicle (DMSO) concentration was kept constant at 0.05% in the bath solution throughout the experiments. In addition, a 23-nl aliquot of calphostin C solution (200 nM, an irreversible inhibitor of protein kinase C) or 0.05% DMSO was injected with an autoinjector (Nanoject; Drummond Scientific Company, Broomall, PA) into oocytes expressing nicotinic receptors at least 30 min before measuring ACh-elicited current, as described previously (Gopalakrishna et al., 1992). The effect of long-term choline treatment on the α7 nicotinic receptors was examined by applying choline 6 min before measuring the current elicited by short pulses of ACh (1 mM, 0.2 s).

Concentration-response curves for agonists were fitted by the equation $I = I_{max}/[1 + (EC_{50}/A)^{nH}]$, whereas concentration-inhibition curves for antagonists were fitted by the equation $I = I_{max} - I_{max}/[1 + (IC_{50}/An)^{nH}]$, using Prism (GraphPad Software, Inc., San Diego, CA), in which I is the percentage amplitude, I_{max} is the maximal response normalized to the current evoked by 1 mM ACh or 10 mM choline (in the absence of the antagonist for inhibitory curves), A is the agonist concentration, An is the antagonist concentration, EC_{50} is the agonist concentration eliciting the half-maximum response, IC_{50} is the antagonist concentration eliciting the half-maximal negative response, and n_H is the Hill coefficient. In the present analyses, I_{max} was constrained to 100% and I_{min} was constrained to 0%.

Immunoprecipitation and Western Blotting. After measuring the ACh (1 mM, 5 s)-elicited current in oocytes expressing EGFP-conjugated α7 nicotinic receptors in the presence of PMA, 4α-PMA, or vehicle (0.05% DMSO) for 6 min, the oocytes were immediately homogenized in homogenizing buffer (50 mM Na_2HPO_4, 50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 5 mM benzamidine, 15 mM iodoacetamide, 2 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 100 μg/ml aprotinin, and 1 mM Na_3VO_4). Three oocyte homogenates were mixed in a tube, and membrane-rich fractions were collected by centrifugation (700*g*, 5 min, 4°C) and then solubilized by pipetting in lysis buffer (homogenizing buffer containing 15% SDS). The obtained cell lysates were incubated with an anti-EGFP antibody (Living Colors Full-Length A.v. polyclonal antibody; Clontech) for 18 h at 4°C and then centrifuged for 20 min 12,000*g* at 18°C, and the supernatant was collected to remove cellular debris and mixed with protein G Sepharose (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for 2 h at 18°C. Sepharose resin was collected and then washed three times with lysis buffer. After mixing with Laemmli solution (0.01% bromphenol blue, 50 mM sodium phosphate, 50% glycerol, and 10% SDS) plus 7.5% (±)-dithiothreitol, proteins were denatured in boiling water for 5 min, separated by SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA). The PVDF membrane was blocked for 1 h with 5% nonfat milk in Tris-buffered saline with Tween 20 (10 mM Tris, 150 mM NaCl, and 0.1% Tween 20, pH 7.5) and then incubated with an anti-phosphoserine antibody (QIAGEN, Valencia, CA) or an anti-EGFP antibody for 2 h at 37°C. The PVDF membrane was washed and incubated with horseradish peroxidase-conjugated anti-rabbit IgG (GE Healthcare) at 25°C for 1 h followed by chemiluminescence detection using enhanced chemiluminescence Western blotting detection reagents according to the manufacturer's instructions (GE Healthcare). The relative serine phosphorylation level of α7 nicotinic receptor subunit was calculated as the ratio of the intensity of the band corresponding to phosphorylated α7 to that of the band corresponding to total α7 in each sample.

Statistical Analysis. Data are represented as mean ± S.E.M. unless otherwise indicated. Nonparametric data were analyzed by Fisher's exact test. For parametric data, the significance of differences between two groups was assessed by Student's *t* test, and the significance of differences between multiple groups were assessed by one-way analysis of variance followed by Scheffé's multiple comparison test. Values of P less than 0.05 were considered to be significant.

Results

Identification of Nonsynonymous Variant in Human *CHRNA7* Gene. We performed polymorphism screening of the *CHRNA7* gene in 100 patients with schizophrenia and 100 control subjects by PCR-SSCP and sequence analyses. In the 231-bp core promoter region of the *CHRNA7* gene, only one SNP was identified at 194 bp upstream from the ATG start codon (−194 G to C; Table 2). Sixteen of the 100 patients with schizophrenia and 20 of the 100 control subjects had this polymorphism, and allele frequencies were not significantly different between the two groups.

Next, we searched for polymorphisms at the coding region and exon-intron boundaries of the *CHRNA7* gene. From exon 1 to exon 4, no SNP was detected in either group. *CHRNA7* exons 5 to 10 are duplicated in the *CHRFAM7A* gene (Gault et al., 1998). To selectively analyze the *CHRNA7* exons 5 to 10, the primary PCR run was performed before PCR-SSCP analysis on the basis of the difference in the 5′ sequence upstream of exon 5 between the two genes. A single band was observed for every sample, and each PCR product including *CHRNA7* exons 5 to 10 was observed as an expected band of 17.4 kilobase pairs (data not shown). In addition, each PCR

TABLE 2

Allele frequencies of variants identified in the human *CHRNA7* gene

Numbering for exons begins with the A of the ATG start codon as nucleotide +1. Numbering in 5′-UTR and intronic positions is relative either to the 5′ or 3′ boundary of the nearest exon.

Position	Variant	Amino Acid	Allele Frequency	
			Schizophrenic	Control
			n (%)	
5′-UTR	−194 G→C		16/200 (8.0)	21/200 (10.5)
Exon 7	606 C→T		5/200 (2.5)	7/200 (3.5)
Exon 7	690 G→A		18/200 (9.0)	25/200 (12.5)
Exon 9	933 G→A		98/200 (49.0)	99/200 (49.5)
Exon 10	1267 G→A	Gly→Ser	1/200 (0.5)	0/200 (0)
Exon 10	1269 C→T		87/200 (43.5)	85/200 (42.5)
Intron 6	IVS6+11 G→A		49/200 (24.5)	50/200 (25.0)
Intron 7	IVS7+21 C→T		68/200 (34.0)	70/200 (35.0)
Intron 7	IVS8–20 G→A		71/200 (35.5)	73/200 (36.5)
Intron 8	IVS8+29 G→C		1/200 (0.5)	0/200 (0)
Intron 9	IVS9+21 A→G		14/200 (7.0)	11/200 (5.5)
Intron 9	IVS9+37 G→C		92/200 (46.0)	94/200 (47.0)
Intron 9	IVS9+87 C→T		94/200 (47.0)	94/200 (47.0)

UTR, untranslated region; IVS, intronic variant sequence.

product, including *CHRFAM7A* exons 5 to 10, showed the predicted band size of 16.5 kilobase pairs (data not shown). These indicate that the DNA fragments were specifically amplified from either the *CHRNA7* or *CHRFAM7A* gene without producing artifacts or cross-contamination during the PCR. In this region of the *CHRNA7* gene, we identified 12 SNPs (namely, 4 silent SNPs, 1 nonsynonymous SNP, and 7 intronic SNPs) as shown in Table 2. Allele frequencies of these variants were not statistically significantly different between patients with schizophrenia and control subjects. The G-to-A variant at 1267 bp in exon 10 is a nonsynonymous variant with a change of a glycine to a serine at amino acid 423 of the α7 receptor protein, and it was not found in the Single-Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Of 100 patients, 1 was heterozygous for this variant, but none of 100 control subjects possesses the variant (Table 2). In the patient with this nonsynonymous variant, no SNP was found at the same position duplicated in exon 10 of the *CHRFAM7A* gene (data not shown). A variant in intron 8 in the *CHRNA7* gene is also identified as a novel SNP in one of 100 patients with schizophrenia (Table 2).

Effects of Nicotinic Ligands at α7-G423S Mutant Receptors Expressed in *X. laevis* Oocytes. To determine the impact of G423S mutation on the α7 nicotinic receptor function, electrophysiological studies were performed on *X. laevis* oocytes expressing the mutant or wild-type α7 nicotinic receptors. Figure 1A shows current through α7 receptors that decayed very rapidly during application of ACh (0.1–1 mM), as reported previously (Chavez-Noriega et al., 1997). Similar currents were recorded from oocytes expressing α7-G423S receptors. Current in both types of receptor was elicited in the same range of ACh concentrations, and the concentration-response curve for ACh in oocytes expressing α7-G423S receptors (EC_{50} = 0.19 mM) was almost identical with that in oocytes expressing wild-type α7 receptors (EC_{50} = 0.20 mM) (Fig. 1, A and B). In addition, the application of an α7 receptor agonist, choline, also produced inward current through the receptors, and no marked differences in the concentration-response curves for choline were observed between the α7-G423S (EC_{50} = 2.5 mM) and wild-type α7 receptors (EC_{50} = 2.0 mM) (Fig. 1B). When the voltage-dependence of α7-G423S receptor-mediated current elicited by ACh (1 mM) was examined at different holding potentials (from −120 to +20 mV) in 20-mV steps, the voltage-current relationships were identical with those of wild-type receptor-mediated current (Fig. 1C). Furthermore, a selective α7 receptor antagonist, MLA, equally blocked the responses mediated by α7-G423S (IC_{50} = 0.21 nM) and wild-type α7 receptors (IC_{50} = 0.22 nM). These results demonstrate that the G423S mutation does not affect the responses of α7 receptors to nicotinic ligands.

Decrease in α7-G423S Nicotinic Receptor Activity by Protein Kinase C Activator. Because the G423S mutation of the α7 receptors is located in the second intracellular loop between the third (M3) and the fourth transmembrane segment (M4), it might change the mode of interaction between the α7 receptors and certain intracellular molecules such as serine/threonine protein kinases. To investigate this hypothesis, we measured ACh (1 mM)-elicited current through the wild-type and mutant α7 receptors in the presence of a protein kinase C activator, PMA. After 6 min of treatment with PMA (10 nM), which by itself did not evoke current, the peak amplitude of α7-G423S receptor-mediated current significantly decreased, whereas no obvious change in wild-type α7 receptor-mediated current was observed following the treatment with PMA (Fig. 2A). This effect of PMA on α7-G423S current persisted during 21 min of observation (Fig. 2A). Similar effects were observed at a higher concentration of PMA (30 nM): after the treatment with PMA (30 nM) for 6 min, the peak amplitude of α7-G423S current decreased to 71 ± 7% (n = 4), whereas that of α7 current remained unaltered (102 ± 7%, n = 5). In contrast, 4α-PMA (10 nM), an inactive enantiomer of PMA, did not affect α7 or α7-G423S receptor responses (Fig. 2B). After 6 min of treatment with 4α-PMA (30 nM), the peak amplitudes of α7 current and α7-G423S current were 100 ± 13% (n = 6) and 97 ± 4% (n = 4), respectively.

When an irreversible inhibitor of protein kinase C, calphostin C, was injected into oocytes expressing α7-G423S receptors, the subsequent treatment with PMA (10 nM) did not inhibit α7-G423S current (Fig. 3, A and B). When the vehicle (0.05% DMSO) was injected instead of calphostin C, treatment with PMA (10 nM) decreased the peak amplitude

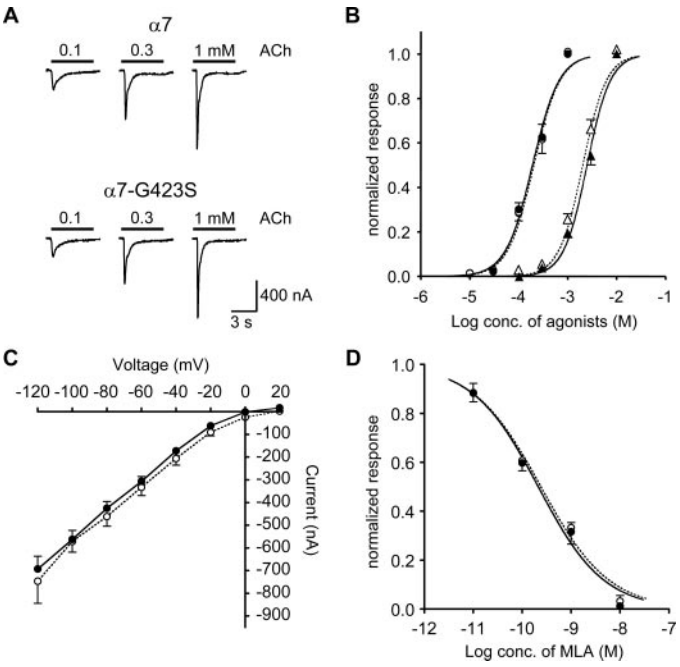

Fig. 1. Comparison of responses to nicotinic ligands between α7 and α7-G423S nicotinic receptors expressed in *X. laevis* oocytes. Current was recorded in the voltage-clamp mode at −60 mV unless otherwise indicated. A, traces showing typical recordings of α7 and α7-G423S receptor-mediated current elicited by ACh. Horizontal bars above the traces indicate the period (5 s) of perfusion with ACh at concentrations indicated. B, concentration-response curves for ACh in oocytes expressing α7 (○) or α7-G423S receptors (●) and for choline in oocytes expressing α7 (△) or α7-G423S receptors (▲). Each current response was normalized to the current evoked by 1 mM ACh or 10 mM choline recorded in the same oocyte. C, current-voltage relationship in oocytes expressing α7 (○) and α7-G423S receptors (●). Oocytes were voltage-clamped at various membrane potentials (from −120 to +20 mV) in 20-mV steps. Current was elicited by ACh (1 mM, 5 s) at different holding potentials. D, curves showing normalized response in terms of ACh (1 mM, 5 s)-induced current in oocytes expressing α7 (○) and α7-G423S receptors (●) against MLA concentration. For test responses, oocytes were preincubated with MLA for 6 min and then exposed to ACh with MLA. The test responses were normalized to the current recorded before the application of MLA (control responses) in the same oocytes. Values represent the mean ± S.E.M. of 5 to 10 separate experiments.

of α7-G423S current (Fig. 3, A and B), the extent of which was similar to that shown in Fig. 2A. These results demonstrate that PMA-induced inhibition of α7-G423S receptor-mediated responses was blocked by calphostin C.

Enhancement of α7-G423S Receptor Desensitization by Protein Kinase C Activator. It was shown previously that nicotinic acetylcholine receptors undergo desensitization during repeated or continuous exposure to an agonist (Quick and Lester, 2002; Giniatullin et al., 2005). To determine the desensitization characteristics of α7-G423S receptors, the effect of repetitive ACh stimulation was examined. When oocytes expressing α7 receptors were stimulated with short pulses of ACh (1 mM, 0.1 s) at 0.17 or 0.33 Hz, the current amplitude progressively declined in a frequency-dependent manner: current amplitudes decreased by 12 ± 3% and 74 ± 5% after 30 s of low- and high-frequency stimulations, respectively (Fig. 4A). The same oocytes were subsequently treated with PMA (10 nM) for 6 min, and then current in response to the repetitive pulses of ACh coapplied with PMA was measured. In this case, α7 current gradually decreased as similarly observed in the absence of PMA (Fig. 4A). When oocytes expressing α7-G423S receptors were stimulated with ACh pulses at 0.17 or 0.33 Hz, a progressive rundown of current was induced, comparable with that observed for α7 current: the current amplitudes decreased by 12 ± 3% and 72 ± 3% after 30 s of low- and high-frequency stimulations, respectively (Fig. 4B). In the presence of PMA (10 nM), however, the rundown of α7-G423S receptor-mediated current was significantly more pronounced, and the peak amplitudes of current elicited by ACh pulses at 0.17 and 0.33 Hz decreased by 43 ± 7% and 83 ± 3%, respectively, after 30 s of stimulation (Fig. 4B).

Long-term exposure to low concentrations of nicotinic agonists, including choline, promotes desensitization of α7 receptors (Papke et al., 1996). To determine whether choline desensitizes α7-G423S receptors and α7 receptors, we compared the effect of long-term exposure to various concentrations of choline between these two types of receptor. The activities of the receptors were assessed by short pulses of ACh (1 mM, 0.2 s) to avoid the desensitization caused by the ACh challenge. As shown in Fig. 5A, continuous exposure to choline for 6 min resulted in a concentration-dependent inhibition of ACh-elicited current through α7 and α7-G423S receptors. No obvious differences in concentration-inhibitory response curves for choline were observed between them (IC_{50} = 83.6 μM at α7; IC_{50} = 82.5 μM at α7-G423S). Furthermore, to compare the effect of PMA on the long-term choline-induced inhibition of the receptors, oocytes expressing α7 or α7-G423S receptors were preincubated with PMA (10 nM) for 6 min and then incubated with a low concentration (30 μM) of choline plus PMA (10 nM) for another 6 min. Under these conditions, the α7-G423S receptor-mediated current in response to ACh decreased by 22% after the 6-min treatment with choline, whereas the wild-type receptor-mediated current was not significantly affected by this treatment (Fig. 5B). These results demonstrate that the treatment with PMA selectively altered the desensitization properties of α7-G423S receptors.

Phosphorylation of α7-G423S Receptors by Protein Kinase C-Dependent Mechanism. We further investigated whether α7-G423S nicotinic receptors are phosphorylated after treatment with PMA. To this end, we prepared oocytes expressing EGFP-fused α7 ($\alpha7^{EGFP}$) receptors and EGFP-fused α7-G423S ($\alpha7^{EGFP}$-G423S) receptors to specifically detect the α7 subunit by Western blotting (see *Discussion*). At first, we examined the electrophysiological properties of $\alpha7^{EGFP}$ and $\alpha7^{EGFP}$-G423S receptors by measuring ACh (1 mM, 5 s)-elicited current. We observed no marked changes in the fast-decaying property and absolute peak amplitude of current compared with untagged receptors

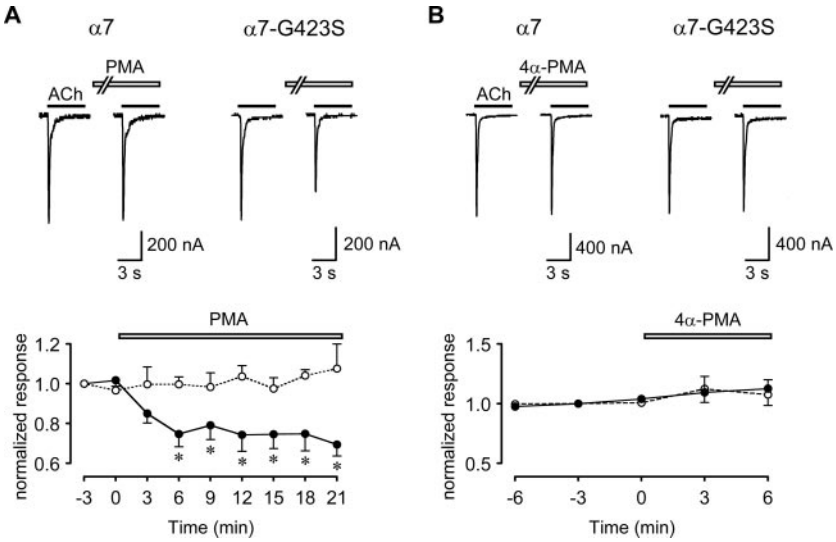

Fig. 2. Attenuation of α7-G423S nicotinic receptor responses after treatment with protein kinase C activator, PMA. Current elicited by ACh (1 mM, 5 s) was recorded every 3 min in the voltage-clamp mode at −60 mV. A, top, typical recordings of ACh-elicited current in oocytes expressing either α7 or α7-G423S receptors before and 6 min after treatment with PMA (10 nM). Horizontal open bars and solid bars indicate the periods of perfusion with PMA and ACh, respectively. Bottom, plots of the peak amplitudes of ACh-elicited currents in oocytes expressing α7 (○) and α7-G423S receptors (●) before and during application of PMA (10 nM). Each current response was normalized to the current recorded 3 min before application of PMA in the same oocyte. B, top, typical recordings of α7 and α7-G423S receptor-mediated current before and 6 min after treatment with 4α-PMA (10 nM), an inactive analog of PMA. Bottom, plots of peak amplitudes of α7 (○) and α7-G423S receptor-mediated current (●) before and during application of 4α-PMA (10 nM). Each response was normalized to the current recorded 3 min before application of 4α-PMA in the same oocyte. Values represent the mean ± S.E.M. of four to seven separate experiments. *, $P < 0.05$ compared with the current recorded in the absence of PMA at 0 min.

(data not shown). Moreover, the inhibitory effect of PMA (30 nM) was also observed on $\alpha7^{EGFP}$-G423S current but not on $\alpha7^{EGFP}$ current (Fig. 6, A and B): by the treatment with PMA (30 nM) for 6 min, the peak amplitude of $\alpha7^{EGFP}$-G423S currents decreased to 70 ± 8% (n = 7), whereas that of $\alpha7^{EGFP}$ current was unaffected (101 ± 3%, n = 3). In contrast, 4α-PMA (30 nM) had no effect on $\alpha7^{EGFP}$ or $\alpha7^{EGFP}$-G423S current: 6 min after treatment with 4α-PMA (30 nM), the peak amplitudes of $\alpha7^{EGFP}$ current and $\alpha7^{EGFP}$-G423S current were 102 ± 5% (n = 3) and 105 ± 2% (n = 7), respectively.

We further investigated the phosphorylation levels in $\alpha7^{EGFP}$ and $\alpha7^{EGFP}$-G423S proteins, which were purified by immunoprecipitation with the anti-EGFP antibody and labeled with either the anti-phosphoserine antibody or the anti-EGFP antibody. As expected from the molecular size of the $\alpha7^{EGFP}$ receptor subunit (57 kDa α7 subunit plus 27 kDa EGFP), the $\alpha7^{EGFP}$ protein was recognized by the anti-EGFP antibody and observed as a major band of 84 kDa, but no band was detected in the lane of $\alpha7^{EGFP}$ receptors treated with vehicle, PMA (30 nM), or 4α-PMA (30 nM) and labeled with the anti-phosphoserine antibody (Fig. 6C). It is interesting that the band corresponding to an 84-kDa protein was observed in the lane of $\alpha7^{EGFP}$-G423S receptors treated with PMA (30 nM) and labeled with the anti-phosphoserine antibody, whereas no such band was observed in the lane of $\alpha7^{EGFP}$-G423S receptors treated with vehicle or 4α-PMA (30 nM) (Fig. 6D). Relative phosphorylation levels in the presence of PMA were significantly higher than the control levels (data not shown). These results indicate that treatment with PMA selectively increased the serine phosphorylation level in $\alpha7^{EGFP}$-G423S receptors.

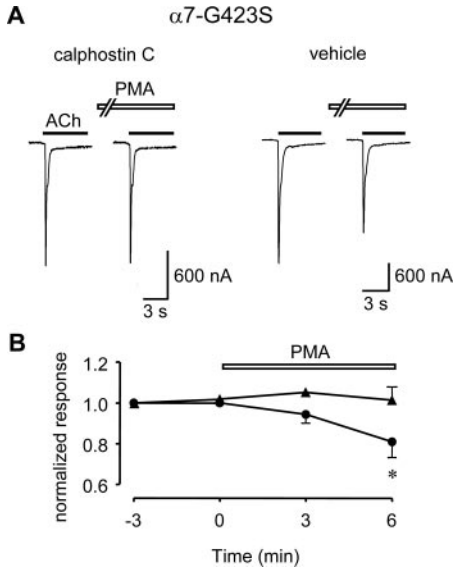

Fig. 3. Protein kinase C inhibitor calphostin C blocks PMA-induced attenuation of α7-G423S receptor responses in *X. laevis* oocytes. Calphostin C (200 nM) or vehicle (0.05% DMSO) was injected into oocytes expressing α7-G423S receptors. Current elicited by ACh (1 mM, 5 s) was recorded every 3 min in the voltage-clamp mode at −60 mV. A, typical traces showing the effect of PMA (10 nM) on α7-G423S receptor-mediated current in oocytes preinjected with calphostin C or vehicle (DMSO). Horizontal open bars and solid bars indicate the periods of perfusion with PMA and ACh, respectively. B, plots of the peak amplitude of α7-G423S receptor-mediated current before and during application of PMA (10 nM) in oocytes preinjected with calphostin C (▲) or vehicle (●). Each response was normalized to the current recorded 3 min before application of PMA in the same oocyte. Values represent the mean ± S.E.M. of 7 to 10 separate experiments. *, $P < 0.05$ compared with the current recorded in the absence of PMA at 0 min.

Discussion

Site-directed mutagenesis studies have shown that single amino acid substitutions in drug receptors, including the α7 nicotinic acetylcholine receptors, are often sufficient to cause marked changes in receptor properties (Galzi and Changeux, 1995). To date, a limited number of nonsynonymous variants have been identified in the human *CHRNA7* gene (Gault et al., 2003), and there is no report evaluating the functional significance of the variants. In the present polymorphism screening, we identified 13 SNPs in the *CHRNA7* gene, including 2 novel SNPs: one was a novel SNP (1267 G to A) within exon 10, and the other was intronic. In particular, the novel coding SNP results in an amino acid substitution from glycine to serine at position 423 located in the large intracellular loop of the α7 receptor subunit. This glycine residue is conserved across several species, namely, human (GenPept accession number NP_000737), rhesus monkey (*Macaca mulatta*, NP_001028055), mouse (NP_031416), and rat (NP_036964), suggesting that there is evolutionary pressure for the protein sequences to maintain normal receptor function. Because statistical analyses could not reveal the direct association of the SNPs identified in this study with schizophrenia, the neurological significance of the nonsynonymous 1267A variant remains to be determined. Hence, additional studies in other cohorts with large-scale populations are needed to clarify whether this rare nonsynonymous variant is, in fact, associated with some central cholinergic disorder.

To evaluate functional consequences of the G423S mutation, we first investigated the effects of nicotinic ligands on mutant-receptor-mediated current. Compared with the responses of wild-type α7 receptors, α7-G423S receptors exhibited normal responses to the nicotinic agonists ACh and choline and to the α7 antagonist MLA. In addition, the α7 and α7-G423S receptors showed a nearly identical current-voltage relationship. These results suggest that the G423S mutation does not affect the receptor-channel opening upon agonist binding, probably because this mutant amino acid is located in an intracellular region that is not functionally related to the ACh-binding site in the extracellular N-terminal domain and to the M2 transmembrane segment that lines the channel pore.

Protein phosphorylation is considered an important mechanism of regulating the α7 nicotinic receptor function, because the tyrosine phosphorylation of α7 receptors by Src-family kinases negatively regulates receptor activity in SH-SY5Y neuroblastoma cells, *X. laevis* oocytes, and rat hippocampal interneurons (Charpantier et al., 2005). In addition, cAMP-dependent protein kinase (protein kinase A) directly phosphorylates the serine 342 residue of rat and chick α7 receptors (Moss et al., 1996), which is also conserved in human α7 receptors (Wecker et al., 2001), and treatment with a cAMP analog enhances calcium influx through rat α7 receptors stably expressed in GH_4C_1 pituitary cells (Quik et al., 1997). Neither protein kinase C nor calcium/calmodulin-dependent protein kinase II can directly phosphorylate rat and chick α7 receptors (Moss et al., 1996). Therefore, we next investigated the possibility that the G423S mutation alters

the mode of interaction between the α7 receptors and certain serine/threonine protein kinases. We were surprised to find that α7-G423S receptor-mediated current markedly decreased in the presence of a phorbol ester, PMA, whereas α7 current was unaffected, consistent with previous reports (Quik et al., 1997; Filippova et al., 2000). The observations that the inhibitory effect of PMA on α7-G423S current was reversed by a protein kinase C inhibitor, calphostin C, and that 4α-PMA caused no such inhibitory effect, strongly suggest that PMA causes the decrease via protein kinase C activation. Thus, the G423S mutation may provide a new site of interaction between the α7 receptors and protein kinase C, although we cannot rule out the possibility that some downstream kinases in the protein kinase C-signaling pathway mediate the effect of PMA. Further studies would be required to determine whether the mutant receptors interact with other serine/threonine protein kinases.

To elucidate whether the phosphorylation of the mutant α7 nicotinic receptors at serine 423 underlies the altered effect of PMA, serine phosphorylation was assessed by Western blotting. Because it has been shown that the most commonly used antibodies to the α7 receptor subunit unexpectedly exhibit equal immunoreactivity in tissues of both wild-type and α7-knockout mice, the labeling of epitope-tagged α7 receptors is now considered to be the most reliable method of identifying α7 immunoreactivity (Jones and Wonnacott, 2005). We therefore prepared EGFP-fused wild-type or mutant α7 receptors expressed in *X. laevis* oocytes. Similar EGFP-fused α7 receptors were reported previously to exhibit normal ACh sensitivity and normal single-channel conductance, although the fusion of EGFP to the receptor lengthens the channel open time (Fucile et al., 2002; Palma et al., 2002). In our present electrophysiological recordings, we observed that the fusion of EGFP to the wild-type and mutant α7 receptors did not change their responses to PMA and 4α-PMA. It is noteworthy that under the condition that α7-G423S current was selectively decreased by PMA, serine phosphorylation level significantly increased in the mutant receptors but not in the wild-type receptors. These results suggest that the mutant α7 receptors are directly phosphorylated at serine 423 by a protein kinase C-dependent mechanism. In the hippocampus, high-frequency stimulation induces long-term potentiation of synaptic transmission, which underlies learning and memory formation, and the induction of long-term potentiation generates a prolonged activation of protein kinase C (Soderling and Derkach, 2000). Therefore, we suggest that the α7-G423S mutant receptors may be phosphorylated via protein kinase C under certain physiological conditions at hippocampal excitatory synapses.

The role of phosphorylation in desensitization of muscle nicotinic receptors has been studied intensively (Swope et al., 1992). For example, the direct phosphorylation of the δ subunit by protein kinase C, or the γ and δ subunits by protein kinase A, is associated with an increase in the rate of nicotinic receptor desensitization. Likewise, the onset of desensitization of ganglionic nicotinic receptors is promoted by phorbol ester activation of protein kinase C (Quick and Lester, 2002). However, there is no evidence at present that phosphorylation modulates the kinetics of desensitization of α7 nicotinic receptors. In this study, we found that the treat-

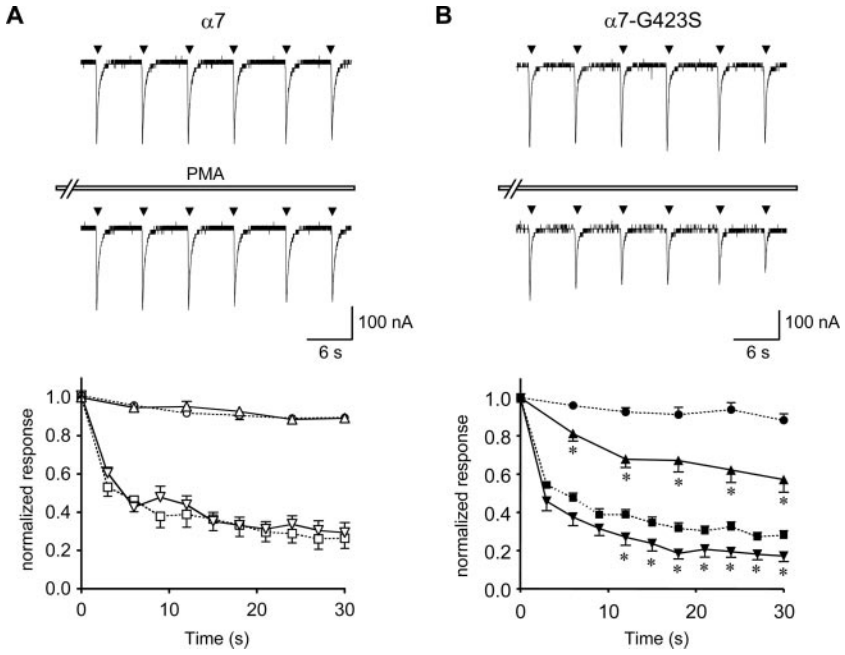

Fig. 4. PMA promotes desensitization of α7-G423S nicotinic receptors induced by repetitive ACh stimulation. Oocytes expressing α7 or α7-G423S receptors were voltage-clamped at −60 mV, and the current elicited every 3 s (at 0.33 Hz) or 6 s (at 0.17 Hz) by short pulses of ACh (1 mM, 0.1 s) was recorded. Thereafter, PMA (10 nM) was superfused in the bath for 6 min, then the responses to the repetitive pulses of ACh coapplied with PMA (10 nM) were recorded in the same oocyte. A, top, typical recordings of the α7 current elicited every 6 s by the ACh pulses (arrowheads) in the absence or presence of PMA (10 nM). Horizontal open bars indicate the period of perfusion with PMA. Bottom, plot of the peak amplitudes of α7 currents elicited by repetitive ACh pulses before (○, 0.17 Hz; □, 0.33 Hz) and during application of 10 nM PMA (△, 0.17 Hz; ▽, 0.33 Hz). Each response was normalized to the current elicited by the initial ACh pulse. B, top, typical recordings of α7-G423S receptor-mediated current elicited every 6 s by repetitive pulses of ACh in the absence or presence of PMA (10 nM). Bottom, plot of peak amplitudes of α7-G423S current elicited by ACh pulses before (●, 0.17 Hz; ■, 0.33 Hz) and during application of 10 nM PMA (▲, 0.17 Hz; ▼, 0.33 Hz). Values represent the mean ± S.E.M. of 10 to 11 separate experiments. ∗, $P < 0.05$ compared with responses in the absence of PMA at each frequency.

ment with PMA promoted the onset of desensitization of the α7-G423S mutant receptors when there was repetitive stimulation with ACh pulses or prolonged exposure to choline. In contrast, we observed that the onset of desensitization of wild-type α7 receptors was unaffected by treatment with PMA and was equivalent to that of the α7-G423S mutant receptors in the absence of PMA. Together with the present analyses of protein phosphorylation, it is possible that phosphorylation of serine 423 residue of the mutant receptors plays an important role in enhancing the receptor desensitization, whereas undetectable levels of serine phosphorylation of wild-type receptors in the absence and presence of PMA and the mutant receptors in the absence of PMA, if any, do not contribute to the enhancement of desensitization. Thus, it is likely that α7 nicotinic receptors bearing G423S substitution acquire a new desensitization mechanism through protein kinase C.

The α7 nicotinic receptors have unique pharmacological properties, compared with other neuronal nicotinic receptors in the mammalian brain, including high Ca^{2+} permeability comparable with that of the *N*-methyl-D-aspartate (NMDA) receptors (Gotti and Clementi, 2004). In addition, current through nicotinic receptors exhibits voltage-dependence, which differs from that of voltage-operated Ca^{2+} channels and NMDA receptors. At hyperpolarized potentials, nicotinic receptors pass current effectively, whereas voltage-operated Ca^{2+} channels and NMDA receptors are inactivated (Dani, 2001). Consequently, Ca^{2+} influx through α7 receptors exerts unique effects, such as the promotion of neuronal survival (Gotti and Clementi, 2004). If the α7 receptors are intensively desensitized because of the G423S mutation, the loss of function may not be fully compensated by other Ca^{2+} channels coexpressed in neurons. The degree and types of changes in neural functions by possessing the α7-G423S mutant receptors need to be clarified in a future study.

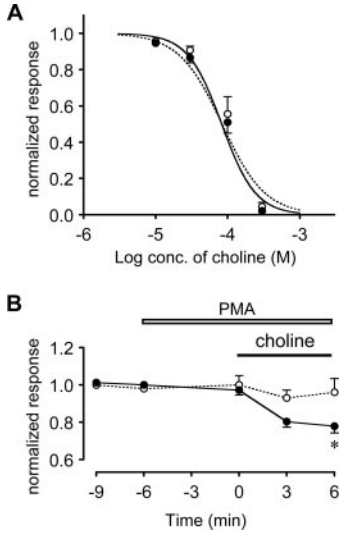

Fig. 5. PMA enhances the desensitization of α7-G423S nicotinic receptors induced by prolonged exposure to choline. Current elicited by short pulses of ACh (1 mM, 0.2 s) was recorded every 3 min in the voltage-clamp mode at −60 mV. A, curves showing normalized response in terms of ACh-elicited current through α7 (○) and α7-G423S receptors (●) expressed in *X. laevis* oocytes against choline concentration. Oocytes were preincubated with choline for 6 min and then stimulated with ACh pulses. Each response was normalized to the current recorded before the application of choline in the same oocyte. Values represent the mean ± S.E.M. of five to seven separate experiments. B, time course of desensitization of α7 (○) and α7-G423S receptors (●) induced by long-term exposure to choline (30 μM) in the presence of PMA (10 nM). Oocytes were preincubated with PMA and then treated with choline plus PMA for 6 min. Current elicited by ACh coapplied with PMA and choline in each oocyte was normalized to the current recorded before application of PMA (control responses) in the same oocyte. Values represent the mean ± S.E.M. of 10 separate experiments. *, $P < 0.05$ compared with the responses to ACh coapplied with PMA (in the absence of choline) at 0 min.

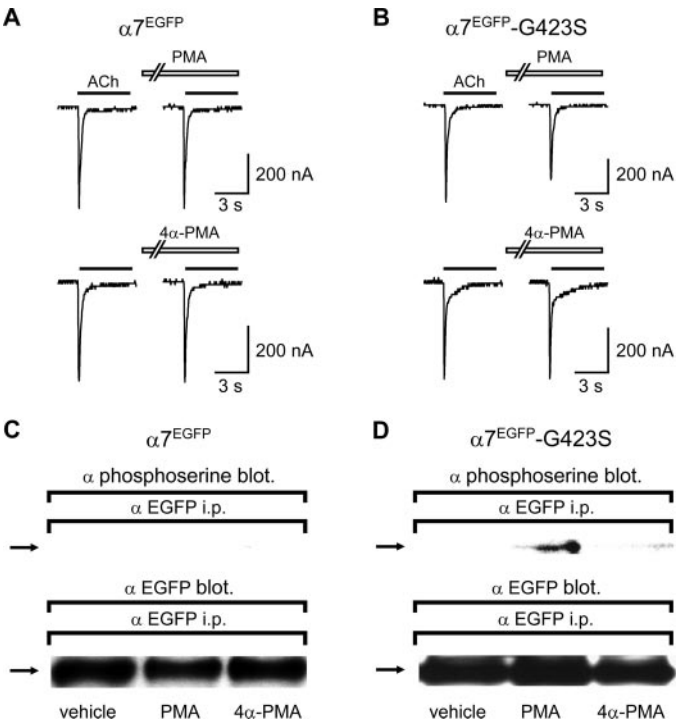

Fig. 6. PMA increases serine phosphorylation level of α7-G423S nicotinic receptors in *X. laevis* oocytes. The oocytes expressing EGFP-fused α7 ($\alpha7^{EGFP}$) receptors and EGFP-fused α7-G423S ($\alpha7^{EGFP}$-G423S) receptors were treated with PMA (30 nM) or 4α-PMA (30 nM) for 6 min, and ACh (1 mM, 5 s)-elicited currents were recorded in the voltage-clamp mode at −60 mV. Subsequently, the oocytes were lysed, and EGFP-fused receptor proteins (84 kDa) were immunoprecipitated with the anti-EGFP antibody and analyzed by Western blotting. Traces show typical recordings of ACh (1 mM, 5 s)-elicited current in oocytes expressing either $\alpha7^{EGFP}$ (A) or $\alpha7^{EGFP}$-G423S receptors (B) at −60 mV before and 6 min after treatment with PMA or 4α-PMA. Horizontal open bars indicate the presence of PMA or 4α-PMA, and horizontal solid bars indicate the period of perfusion with ACh. Immunoblots show bands corresponding to the 84-kDa protein recognized by the anti-phosphoserine antibody or anti-EGFP antibody in samples from oocytes expressing $\alpha7^{EGFP}$ (C) and $\alpha7^{EGFP}$-G423S receptors (D). Similar results were observed in three separate experiments.

In summary, we found a novel SNP in the *CHRNA7* gene in a Japanese population, which results in G423S mutation of the α7 nicotinic receptors. We also demonstrated that the G423S mutation promotes receptor desensitization by a protein kinase C-dependent mechanism. To the best of our knowledge, this is the first study demonstrating that human gene mutation contributes to the dysfunction of the α7 nicotinic receptors.

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