tein kinase (MAPK) pathway. However, we found no alteration of the pErk1/2 IR level in the hippocampus. In contrast, Ujike *et al.*[9] reported that chronic methamphetamine may activate selected MAPKs in restricted regions, that is, the novel MAPK cascade in the frontal cortex and the classic MAPK cascade in the hippocampus.

A recent set of studies suggested that the Erk/MAPK pathway plays a fundamental role in vertebrate memory consolidation. MAPK is specifically activated by the associative conditioning that induces memory consolidation rather than by an aversive stimulus.[20,21] Of interest, stimulation of the hippocampus (ventral subiculum) triggers the memory that is integral to craving.[22] Craving is also associated with activation of memory circuits, including the amygdala (implicated in conditioned learning), hippocampus (implicated in declarative learning), and dorsal striatum (implicated in habit learning), all of which receive DA innervation (FIG. 3).[23] Thus, it is necessary to focus on the role of period genes in the hippocampus and to make further studies on methamphetamine-induced sensitization and reward.

Our experiments were performed according to the "Standardization of Protocols in cDNA Microarray Analysis" by Benes and Muckenthaler,[12] and our cDNA microarray system is able to reliably detect a 1.4-fold change in mRNA content. The cDNA microarray represents an invaluable tool for the identification of gene alterations at the mRNA level, and the identification of substance-abuse–sensitive genes should provide a key to elucidate the molecular mechanisms involved in the process of drug abuse and contribute to the discovery of biomarkers for early detection and diagnosis of such abuse, in addition to the development of novel addictive drugs.

ACKNOWLEDGMENTS

This work was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#15700300), and by a grant from the Ministry of Health, Labour, and Welfare of Japan (#H14-toxico-011), and by grants from RIKEN-BSI, from Toyota Phys. & Chem. Res. Inst., and the National Center for Neurology and Psychiatry (15-3-03). We are grateful to Dr. Keiko Matsuoka for her helpful assistance. We also thank Ms. Harumi Hata for her excellent technical assistance.

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A Possible Genetic Mechanism Underlying Individual and Interstrain Differences in Opioid Actions

Focus on the Mu Opioid Receptor Gene

WENHUA HAN,[a] SOICHIRO IDE,[a,b] ICHIRO SORA,[a,c] HIDEKO YAMAMOTO,[a] AND KAZUTAKA IKEDA[a]

[a]*Department of Molecular Psychiatry, Tokyo Insititute of Psychiatry, Tokyo 156-8585, Japan*

[b]*Department of Molecular Pharmacology, Faculty of Pharmaceutical Science, Kyoto University, Kyoto 606-8501, Japan*

[c]*Department of Neuroscience, Division of Psychobiology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan*

ABSTRACT: **Individual differences in responses to opioids limit effective pain treatment with these drugs. Identifying the mechanism could help to improve the analgesic effects of them. Since the molecular cloning of the mu opioid receptor (muOR) gene, substantial advances in opioid research have been made, including the discoveries that muOR plays a mandatory role in the analgesic effects of opioids and that the sequence of the muOR gene varies from one individual to another. It is conceivable that the differences in the muOR gene cause individual differences in opioid actions. The present review summarizes the recent advances made in research on human and mouse muOR genes and proposes that the variances in the 3′ untranslated region (3′-UTR) of the muOR gene might participate in the variability of the opioid response.**

KEYWORDS: **CXBK mice; muOR gene; opioid; polymorphism; individual differences; 3′-UTR**

INTRODUCTION

Opioids, the oldest and most widely used pain relievers, also have several side effects, including respiratory depression, constipation, tolerance, and addiction. To maximize pain relief and minimize side effects, prescribing the appropriate amount of opioid is very important. However, both clinicians and researchers have observed individual differences in opioid analgesia. Galer *et al.* reported that cancer patients showed individual variability in their response to different opioids,[1] and Levine

Address for correspondence: Kazutaka Ikeda, Department of Molecular Psychiatry, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan. Voice: +81-3-3304-5701, ext. 508; fax: +81-3-3329-8035.
ikedak@prit.go.jp

Ann. N.Y. Acad. Sci. 1026: 370–375 (2004).
doi: 10.1196/annals.1307.045

et al. found that the analgesic effect of morphine on dental extraction pain exhibited individual differences.[2] These individual differences make it difficult to know the suitable dose for each patient. Clarifying the mechanisms contributing to this patient-related variability should help to make drug treatment individualized and to optimize pain relief.

ROLE OF muOR IN OPIOID ANALGESIA

Opioids exert their pharmacological actions through opioid receptors, which exist as mu, delta, and kappa subtypes. Recent studies on mice lacking muOR indicate that, among these three subtypes, muOR is a mandatory component for both endogenous and exogenous opioid actions.[3–5] The basal pain threshold was lowered in muOR knockout (KO) mice,[3] and mice lacking muOR showed no analgesic effect of morphine, although delta and kappa opioid receptors were present.[3,4] Also, the analgesic effects of a delta-opioid receptor agonist were reduced in muOR KO mice.[5,6]

Studies on mice lacking muOR also suggest that morphine analgesia is gene-dose dependent.[3,4] That is, deletion of muOR eliminated the morphine effects on nociception; and heterozygous muOR KO mice, which had 50% of the wild-type receptor density, showed lower but still significant morphine analgesia. These findings suggest that the analgesic effect of morphine is dependent on the amount of the muOR receptor.

INDIVIDUAL DIFFERENCES IN HUMAN muOR GENE

Sequencing of hundreds of individual muOR genes identified two variants in the coding region of the muOR gene: an A118G variant and a C17T variant.[7,8] Uhl *et al.*[9] reported several polymorphisms in the 5′-UTR of human and mouse muOR genes. We have also identified several polymorphisms in the intron 1 and 5′-UTR of the human muOR gene.[10] Variations in coding regions could result in changes in the amino acid sequence. In the case of A118G, the affinity of β-endorphin for the muOR is altered. Variations in untranslated regions (UTRs) could affect the levels of gene expression, mediating the pain-related phenotypes. However, direct evidence that supports the causal relationship between differences in the muOR gene and observed differences in opioid responses has not been reported in humans.

INTERSTRAIN DIFFERENCES AS AN ANALOGY TO INDIVIDUAL DIFFERENCES

Genetic models in animals have been produced and well studied. Inbred- and recombinant inbred (RI)–strain mice provide useful genetic models. These strains are established by sibling mating for at least 20 generations. Whereas the offspring from each strain are virtually identical in genotype and similar in phenotype to each other,[11] mice of different strains show different genotypes and phenotypes. Thus, genetic mechanisms underlying the interstrain differences in mice may be similar to

those operating in humans. Mapping the interstrain genetic variations and revealing the relationship between these variations and differences in phenotypes could aid in clarifying the genetic mechanisms underlying the interstrain and individual differences in responses to opioids.

PHENOTYPES OF CXBK MICE

The CXBK mouse strain, an inbred strain derived from an F_2 intercross between C57BL/6By (B6) and BALB/cBy mice, is a well-studied RI strain.[12] For almost 20 years, this strain has been used as a muOR-deficient mice, because CXBK mice exhibit a deficient morphine response[13–15] and show decreased opioid binding.[13] We have demonstrated that CXBK mice display a phenotype similar to that of heterozygous muOR KO mice.[14,15] Compared with B6 mice, in which morphine reached its maximal analgesic effect at the dose of 10 mg/kg, CXBK mice only responded to a high dose of morphine (30 or 100 mg/kg).[15]

MOLECULAR MECHANISMS UNDERLYING CXBK MOUSE PHENOTYPES

The sequence of the muOR coding region of CXBK mice was shown to be identical to that of B6 mice,[14] indicating that CXBK mice possess normal muOR protein. By Northern blot analysis, using the muOR coding region as a probe, we detected an estimated 12-kilobase band in B6 mice,[14] just as reported by Kaufman *et al.*[16] However, in CXBK mice, the same probe detected an estimated 14.5-kilobase band, the intensity of which was about half of that of the band in B6 mice.[14] This result is in accord with the reported decrease in [^{3}H]naloxone binding in CXBK mice.[13] The reduced muOR mRNA level would be the cause of the reduced morphine antinociception in CXBK mice, as in the case of heterozygous muOR KO mice.

Testing of inbred descendants of an F_2 intercross between B6 and CXBK mouse strains confirmed that both the altered pain phenotype and muOR mRNA expression were related to the muOR gene.[14] In Northern blot analyses, samples from mice inheriting two copies of the B6 mouse gene (B6μ) gave a discrete band corresponding to B6 mouse muOR mRNA, and those from mice inheriting two copies of the CXBK mouse gene (CXμ) showed a band corresponding to CXBK mouse muOR mRNA. For samples from mice inheriting one copy of the B6 mouse gene and one copy of the CXBK mouse gene (Heμ), both bands were seen.

In agreement with the results from the Northern blot analysis, in the hot plate test, the morphine analgesia in the Heμ mice was higher than that in the CXμ mice, but lower than that in the B6μ ones.

Recently we identified an approximate 5-kilobase insertion in the 3′-UTR of the CXBK mouse muOR gene (unpublished data). This inserted sequence would account for the longer than normal mRNA. As discussed below, the extended 3′-UTR may lower the muOR mRNA translation rate and/or stability and, thus in turn, reduce the level of muOR protein and the opioid analgesic effects in CXBK mice.

3′-UTR AND GENE EXPRESSION

A recent computational analysis of a large UTR database[17] revealed that in humans, the mean length of 3′-UTRs is nearly four times longer than that of 5′-UTRs. Further analysis of the same database suggests that the length of 3′-UTRs has increased during evolution, whereas the mean length of 5′-UTRs has been conserved in organisms ranging from fungi and plants to invertebrates and vertebrates, including humans. The extended 3′-UTRs may play a role in the regulation of gene expression.

To date, there is no evidence indicating that mutations of the human muOR 3′-UTR alter mRNA expression, consequently contributing to the individual differences in opioid responses. However, several cases of human diseases that are associated with differences in the 3′-UTR of other genes have been reported.[18] For example, Fukuyama-type congenital muscular dystrophy (FCMD), one of the most common autosomal recessive disorders in Japan (incidence is 0.7–1.2 per 10,000 births), is associated with a retrotransposal 3-kilobase insertion within the 3′-UTR of a gene encoding a 461-amino-acid protein called fukutin.[19] Similarly, α-thalassemia is associated with mutations in the 3′-UTR of α-globin mRNA.[20] Furthermore, a polymorphism in the 3′-UTR of human resistin has been shown to correlate with insulin sensitivity.[21]

Although the precise mechanisms of the regulation by 3′-UTRs is largely unknown, recent studies suggest that 3′-UTR mediates mRNA transcription and translation through 3′-UTR sequence-binding proteins.[22–24] Other studies have demonstrated that 3′-UTRs contain stability determinants.[20,22,23] Overall, it has become conceivable that 3′-UTRs can specifically control the rates of translation and degradation of mRNAs.

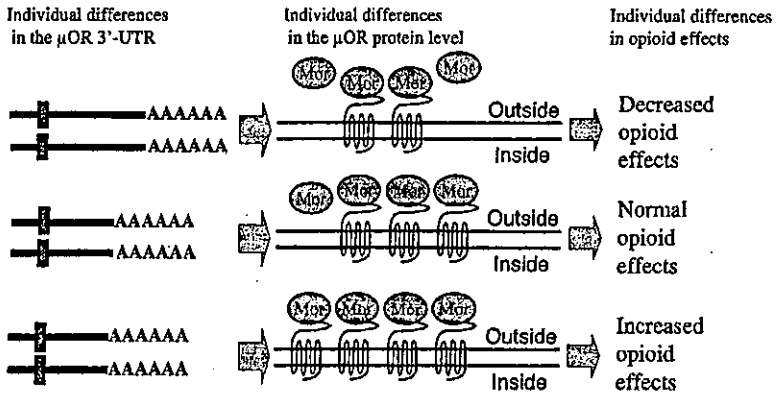

FIGURE 1. Schematic illustration indicating a possible genetic mechanism underlying individual differences in opioid effects. Variation in the 3′-UTR of muOR may cause differences in muOR protein expression and, in turn, result in the reduced opioid responses. Mor, morphine.

A POSSIBLE GENETIC MECHANISM FOR INDIVIDUAL DIFFERENCES IN OPIOID RESPONSES

The insert in the muOR 3′-UTR of CXBK mice likely explains the reduced muOR level and morphine analgesia in these animals. It is also reasonable to assume that the sequences of the 3′-UTR in the human muOR gene are different from individual to individual. Taken together, these variations in the 3′-UTR may be the cause of the individual differences in muOR gene expression and in opioid responses (FIG. 1). This hypothesis will be tested by systematic exploration of polymorphisms in the muOR 3′-UTR and their level of correlation with the pain phenotypes. One day, it may be possible to predict individual morphine sensitivity by muOR gene analysis. Such a technique could optimize drug treatment for each individual.

ACKNOWLEDGMENTS

We thank Ms. Keiko Matsuoka and Ms. Harumi Hata for technical assistance. This work was supported by research grants from MEXT (15700300); MHLW (H14-toxico-011); JSPS (P03584); RIKEN-BSI; Toyota Physical and Chemical Research Institute; and the National Center for Neurology and Psychiatry (15-3-03).

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Modulators of G Protein-Activated Inwardly Rectifying K^+ Channels

Potentially Therapeutic Agents for Addictive Drug Users

TORU KOBAYASHI,[a] KAZUO WASHIYAMA,[a] AND KAZUTAKA IKEDA[b]

[a]*Department of Molecular Neuropathology, Brain Research Institute, Niigata University, Niigata, Niigata 951-8585, Japan*

[b]*Department of Molecular Psychiatry, Tokyo Institute of Psychiatry, Setagaya-ku, Tokyo 156-8585, Japan*

ABSTRACT: **G protein-activated inwardly rectifying K^+ (GIRK, Kir3) channels play an important role in the inhibitory regulation of neuronal excitability in most brain regions and heart rate through activation of various G protein-coupled receptors, such as opioid, cannabinoid, and D_2 dopamine receptors. Therefore, modulators of GIRK channels may affect many brain functions. We have shown using *Xenopus* oocyte expression assays that ethanol directly activates GIRK channels, whereas various antipsychotics (thioridazine, clozapine, pimozide, and haloperidol) inhibit the channels. Here we investigated not only the effects of various selective serotonin reuptake inhibitor (SSRI) antidepressants (fluoxetine, citalopram, fluvoxamine, and zimelidine) and risperidone, an atypical antipsychotic, on GIRK channels, but also those of the various drugs tested on other Kir channels using the *Xenopus* oocyte system. Fluoxetine inhibited GIRK channels, whereas the other SSRIs and risperidone had a small or no effect on the channels. In contrast, Kir1.1 and Kir2.1 channels were insensitive to ethanol and various SSRIs and antipsychotics, although thioridazine weakly inhibited Kir1.1 channels. It has been shown that the function of GIRK channels is involved in seizure susceptibility, antinociception by opioids, cannabinoids, or ethanol, and cocaine reinforcement in studies using GIRK knockout mice and *weaver* mutant mice that have mutant GIRK2 channels insensitive to G proteins and ethanol. Activation of GIRK channels by opioids, cannabinoids, or ethanol may be one of these key effects. Therefore, GIRK channel modulators might be potential agents for the treatment of users of addictive drugs, such as cocaine, opioids, cannabinoids, and ethanol, as well as for the treatment of epilepsy and pain.**

KEYWORDS: **G protein-activated inwardly rectifying K^+ (GIRK, Kir3) channels; Kir channel; selective serotonin reuptake inhibitor; ethanol; antipsychotic drug; *Xenopus* oocyte**

Address for correspondence: Toru Kobayashi, Department of Molecular Neuropathology, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata, Niigata 951-8585, Japan. Voice: +81-25-227-0646; fax: +81-25-227-0818.
torukoba@bri.niigata-u.ac.jp

Ann. N.Y. Acad. Sci. 1025: 590–594 (2004).
doi: 10.1196/annals.1316.073

INTRODUCTION

G protein-activated inwardly rectifying K^+ (GIRK) channels (also known as Kir3 channels) are members of a family of inward-rectifier K^+ (Kir) channels that includes seven subfamilies.[1] Four GIRK channel subunits have been identified in mammals.[1] Neuronal GIRK channels are predominantly heteromultimers composed of GIRK1 and GIRK2 subunits in most brain regions or homomultimers composed of GIRK2 subunits in the substantia nigra and ventral tegmental area, whereas atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits.[1] GIRK channels play an important role in the inhibitory regulation of neuronal excitability in most brain regions and heart rate through activation of various G protein-coupled receptors, such as opioid, cannabinoid, and D_2 dopamine receptors.[1,2] Therefore, modulators of GIRK channels may affect many brain functions. We have shown using *Xenopus* oocyte expression assays that ethanol, at pharmacologically relevant concentrations, directly activates GIRK channels,[3] whereas various antipsychotic drugs (thioridazine, clozapine, pimozide, and haloperidol) inhibit the channels with varying degrees of potency and effectiveness at micromolar concentrations.[4] Recently, we demonstrated that GIRK channels are inhibited by micromolar levels of clinically relevant brain concentrations of fluoxetine, a selective serotonin reuptake inhibitor (SSRI) that has been widely used for the treatment of depression and other psychiatric disorders, including alcoholism.[5] Interestingly, the GIRK currents induced by ethanol were attenuated similarly by fluoxetine.[5] In the present study, we investigated not only the effects of various SSRIs and risperidone, an atypical antipsychotic, on GIRK channels but also those of the various drugs tested on other Kir channels using the *Xenopus* oocyte expression system.

METHODS

For *Xenopus* oocyte expression experiments,[5] *Xenopus laevis* oocytes were injected with mRNA(s) for GIRK1/GIRK2 or GIRK1/GIRK4 combinations, GIRK2, Kir1.1, or Kir2.1. The oocytes were incubated at 19°C in Barth's solution and defolliculated following collagenase treatment. Whole-cell currents were recorded with a conventional two-electrode voltage clamp from the oocytes, which were superfused with a high-potassium solution containing 96 mM K^+. The membrane potential was held at –70 mV.

RESULTS

We first examined the effects of various SSRIs on brain-type GIRK1/2 and GIRK2 channels or cardiac-type GIRK1/4 channels expressed in *Xenopus* oocytes. In contrast to the inhibitory effects of fluoxetine on GIRK channels, citalopram, fluvoxamine, and zimelidine had little or no effect on these GIRK channels (FIG. 1). These results suggest that inhibition of GIRK channels by fluoxetine among SSRIs may contribute to some of its therapeutic effects and adverse side effects. Risperidone slightly inhibited GIRK1/2 and GIRK1/4 channels in a similar manner (1.7 ± 0.6 and 1.6 ± 0.1%; 7.6 ± 1.1 and 6.3 ± 0.9%; and 16.4 ± 1.3 and 14.5 ± 1.7% at 10,

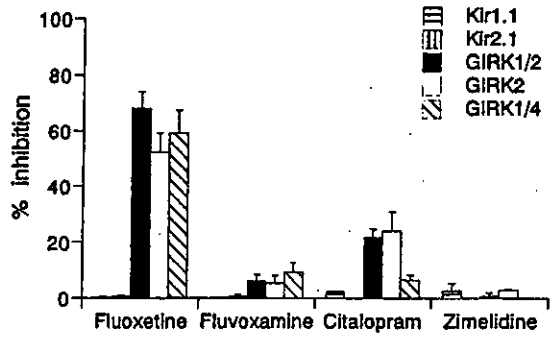

FIGURE 1. Comparison of the SSRI effects on members of inwardly rectifying potassium channels: Kir1.1, Kir2.1, and GIRK channels, expressed as homomeric or heteromeric channels in *Xenopus* oocytes. The concentration of the SSRIs used was 100 μM. Current responses were measured at a membrane potential of −70 mV in a high-potassium solution containing 96 mM K^+ ($N \geq 3$). The magnitudes of inhibition of Kir currents by SSRIs were compared with the current components sensitive to 3 mM Ba^{2+}, which blocked Kir channels. Each value is a mean ± SEM.

30, and 100 μM, $N = 5$, respectively). In contrast to the effects of the various drugs on GIRK channels, Kir1.1 and Kir2.1 channels in other Kir channel subfamilies were insensitive to the SSRIs tested (FIG. 1), ethanol, and various antipsychotic drugs (thioridazine, clozapine, pimozide, haloperidol, and risperidone) (data not shown), although thioridazine inhibited Kir1.1 channels by only 18.1 ± 5.0% at 300 μM ($N = 3$).

DISCUSSION

We have demonstrated that among Kir channels, GIRK channels are modulated by various drugs affecting brain functions, such as ethanol, antipsychotics, and fluoxetine. Furthermore, several agents from diverse chemical and pharmacological classes modulate GIRK channels (TABLE 1).[6–16] These agents may be useful for designing and developing GIRK channel modulators. Clarification of the effects of these agents on GIRK channels *in vivo* may help to advance the understanding of function of GIRK channels. It has been shown that the function of GIRK channels is involved in seizure susceptibility and antinociception by opioids, cannabinoids, or ethanol in studies using GIRK2 knockout mice and *weaver* mutant mice that have mutant GIRK2 channels insensitive to G proteins and ethanol.[1–3,17] GIRK channel activators may be useful for the treatment of epilepsy and pain. In addition, inhibition of GIRK channels by antipsychotics and fluoxetine could lead to an increase in neuronal excitability, suggesting that the inhibition may be related to some effects in clinical practice. The potent inhibition of neuronal GIRK channels may contribute to the cause of seizures in overdoses. Moreover, GIRK knockout mice show decreased cocaine self-administration.[18] Activation of GIRK channels by opioids, can-

TABLE 1. Modulator of GIRK channels

Activator	Reference	Inhibitor	Reference
Ethanol	3	Antipsychotics thioridazine, clozapine, pimozide, haloperidol	4
Flupirtine (non-opioid analgesic)	6	SSRI antidepressant fluoxetine	5
Nitrous oxide (anesthetic)	7	Channel blockers quinidine, verapamil, MK801	10–12
Estrogen	8	Anesthetics F3 (1-chloro-1,2,2-trifluorocyclobutane), halothane, enflurane, isoflurane, bupivacaine	7, 13
Dithiothreitol (reducing agent)	9	Others tertiapin, SCH23390, estrogen, ginsenoside	8, 14–16

nabinoids, or ethanol may be one of these key effects *in vivo*. Therefore, GIRK channel inhibitors might be potential agents for the treatment of users of addictive drugs such as cocaine, opioids, cannabinoids, and ethanol. On the other hand, the withdrawal syndromes of ethanol, opioids, and cannabinoids might be prevented or treated with GIRK channel activators.

ACKNOWLEDGMENTS

We thank Dr. Kansaku Baba for his cooperation, and we thank Tomio Ichikawa, Kazuo Kobayashi, and Kazuyo Sekikawa for their assistance. We also thank Dr. Steven C. Hebert for providing the Kir1.1 cDNA, and Dr. Lily Y. Jan for providing the Kir2.1 cDNA. This work was supported by research grants from the Ministry of Education, Science, Sports, and Culture of Japan; the Ministry of Health, Labour and Welfare of Japan; and the RIKEN Brain Science Institute.

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Gene Polymorphisms of the Mu Opioid Receptor in Methamphetamine Abusers

SOICHIRO IDE,[a,b] HIDEAKI KOBAYASHI,[a] KEIKO TANAKA,[a] HIROSHI UJIKE,[c,l] YOSHIMOTO SEKINE,[d,l] NORIO OZAKI,[e,l] TOSHIYA INADA,[f,l] MUTSUO HARANO,[g,l] TOKUTARO KOMIYAMA,[h,l] MITSUHIKO YAMADA,[i,l] MASAOMI IYO,[j,l] KAZUTAKA IKEDA,[a] AND ICHIRO SORA[a,k,l]

[a]*Department of Molecular Psychiatry, Tokyo Institute of Psychiatry, Tokyo, Japan*

[b]*Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan*

[c]*Department of Neuropsychiatry, Graduate School of Medicine and Dentistry, Okayama University, Okayama, Japan*

[d]*Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Japan*

[e]*Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan*

[f]*Department of Psychiatry and Psychobiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*

[g]*Department of Neuropsychiatry, Kurume University School of Medicine, Kurume, Japan*

[h]*Division of Psychiatry, National Center Hospital for Mental, Nervous, and Muscular Disorders, National Center of Neurology and Psychiatry, Tokyo, Japan*

[i]*Department of Psychiatry, Karasuyama Hospital, Showa University School of Medicine, Tokyo, Japan*

[j]*Department of Psychiatry, Graduate School of Medicine, Chiba University, Chiba, Japan*

[k]*Division of Psychobiology, Department of Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan*

[l]*Japanese Genetics Initiative for Drug Abuse (JGIDA), Japan*

ABSTRACT: **In drug addiction, the opioid system is thought to mediate motivational effects through dopamine-independent mechanisms. We have investigated associations of the μ-opioid receptor gene (OPRM) variations with methamphetamine (MAP) dependence/psychosis. The allelic frequency of A118G (Asn40Asp) in exon 1 of ORPM was 45.3% in our control subjects, but only 7.5–25.8% in the Caucasian or African-American population of previous studies. We have identified several novel polymorphisms in intron 1 and the 5′ untranslated region (5′UTR) of OPRM. Polymorphisms in the functionally rel-**

Address for correspondence: Ichiro Sora, M.D., Ph.D., Division of Psychobiology, Department of Neuroscience, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Sendai, 980-8574, Japan. Voice: +81-22-717-7808; fax: +81-22-717-7809.
isora@mail.tains.tohoku.ac.jp

**Ann. N.Y. Acad. Sci. 1025: 316–324 (2004). © 2004 New York Academy of Sciences.
doi: 10.1196/annals.1316.039**

evant 5′ regulatory region of OPRM were different in our Japanese population from Caucasian or African-American populations. No significant differences between controls and MAP abusers were found in either genotype or allele frequency at any single nucleotide polymorphism (SNP) or (AC)n dinucleotide repeat in intron 1. A subdivision of our MAP group revealed that A118G of OPRM shows a significant association with MAP psychosis having latency less than three years. Further analysis should be capable of identifying associations between the OPRM variations and MAP dependence/psychosis.

KEYWORDS: **single nucleotide polymorphism; nucleotide repeats; human μ-opioid receptor gene; methamphetamine; dependence; psychosis**

INTRODUCTION

Methamphetamines (MAPs) and other psychostimulants produce their effects by potentiating monoaminergic transmission, in which dopamine is believed to be directly related to the reinforcing effect.[1] It is generally believed that dopaminergic nerve systems interact with opioid nerve systems. Opioid receptor agonists regulate dopamine metabolism in nerve endings, regulate dopamine release into the synaptic cleft,[2] and attenuate methamphetamine-induced alterations in dopamine neurotransmission.[3,4] It is therefore possible that variations in the opioid receptor function could give rise—depending on differing susceptibilities among individuals—to the development of MAP dependence and/or psychosis.

Several single nucleotide polymorphisms (SNPs) of the μ-opioid receptor gene (OPRM) that cause amino acid substitution and other SNPs in noncoding regions or silent mutations have been reported, mostly in African-American or Caucasian populations.[5,6] Association between frequencies of polymorphisms in OPRM and opioid, alcohol, or polydrug dependence has been studied in African-American, Caucasian, Hispanic, and Han Chinese populations.[7–14] In the present study, we have screened the coding and functionally relevant regulatory regions of the OPRM for genetic variation in a Japanese population, and examined the association between novel and reported polymorphisms in OPRM and MAP abusers in Japan.

MATERIALS AND METHODS

Study Subjects

The subjects were 138 unrelated patients exhibiting MAP dependence and/or psychotic disorder (107 males and 31 females, average = 35.7 ± 1.1) meeting ICD-10-DCR criteria (F15.2 and F15.5). They were outpatients or inpatients at psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA). As controls we used 213 age-, gender- and geographical origin–matched normal individuals (158 males and 55 females, age 34.4±1.6). Most were medical staff who had no past history and no family history of drug dependence or psychotic disorders. Diagnoses were made by two trained psychiatrists based on an interview and all available information, including hospital records. Patients who had a clinical diagnosis of schizophrenia, another psychotic disorder, or an organic mental syndrome were excluded. All subjects were Japanese, born and living in Japan, including northern

Kyusyu, Setouchi, Tyukyou, Toukai, and Kantou. Advance approval was obtained for this study from the ethics committees of each institute of JGIDA, and all subjects provided written informed consent for the use of their DNA samples in this research.

The patients were divided into two subgroups according to the latency of their psychosis from first MAP intake: less than three years (n = 54, average = 0.83 years) or more than three years (n = 53, average = 9.98 years). For the remaining 31 subjects we were not able to determine the latency period.

Genotyping

Genomic DNA was extracted from a peripheral blood sample using the standard phenol extraction protocol. The 5'UTR and 5' flanking regions (up to about 5.6 kbp from the start codon) of the OPRM were separately amplified by the polymerase chain reaction (PCR) as three fragments. Exon 1 and part of intron 1 of the OPRM were amplified by PCR. To find possible polymorphisms in 5'UTR, exon 1, and part of intron 1 of OPRM, the fragments amplified from genomic DNA of 44 control subjects with PCR were sequenced using BigDye terminators (Applied Biosystems). The genomic DNA of the remaining control subjects and the MAP-dependent subjects was then analyzed by automated DNA sequencing in the region of exon 1 and part of intron 1. Primers were designed based on the reference genomic contingency sequence in the National Center of Biotechnology Information (Genbank Accessions no. NT-023451).

Statistical Analysis

For the statistical analysis, the chi-squared test was used; the statistical significance level was chosen as .05. The Hardy-Weinberg (HW) equilibrium was checked in all polymorphisms using the chi-squared test. In the analysis of linkage disequilibrium and estimation of haplotype frequencies, genotypic data from 179 control subjects and 128 MAP-dependent subjects were analyzed using the Arlequin program available from http://anthro.unige.ch/arlequin.[15] To analyze the variation in nucleotide repeats between control and MAP-dependent subjects, the CLUMP program (16) was used to estimate the significant values of the chi-squared test (T1) based on 1,000 Monte Carlo simulations.

RESULTS

To identify polymorphisms in OPRM, we analyzed exon 1, part of intron 1, and part of 5' UTR of control subjects (n = 44). Seventeen novel and one previously reported polymorphism were found, and there were no deviations from HW expectations (TABLE 1). SNPs (C12G, C17T, G24A) that have already been reported in the exon 1 cording region (5–14) were not identified in our control subjects. The allelic frequency of A118G was remarkably high in our control subjects (45.3%) compared with African-American or Caucasian populations (7.5–25.8%).[7–14] We found that one pair of SNPs, intervening sequence (IVS) 1-A4980G, and IVS1-A4910G in intron 1 were in a relationship of absolute disequilibrium.

We picked up these two SNPs—A118G, which was higher frequency, and IVS1-A4980G, which represented A4980G and A4910G—for analysis of our samples

TABLE 1. List of SNPs found in control subjects (5'UTR, exon 1, and part of intron 1)

Position	SNP Name	Allelic Frequency	Sample Size
5′ flanking region and 5′UTR	A-5580G	<1.5%	44
	C-5342T	<1.5%	44
	A-5308G	<1.5%	44
	C-5236T	<1.5%	44
	G-5026A	<1.5%	44
	G-4936A	<1.5%	44
	C-4504A	<1.5%	44
	T-2694G	<1.5%	44
	A-2693C	<1.5%	44
	T-2683T	<1.5%	44
	T-2402C	<1.5%	44
Exon 1	A118G	<45.3%	213
Intron 1	IVS1-A4980G	2.7%	187
	IVS1-A4910G	2.7%	187
	IVS1-G4690A	<1.5%	113
	IVS1-T4591G	2.7%	113
	IVS1-T4456G	<1.5%	113

(TABLE 2). No significant differences were found in allele frequencies of either SNP between controls and MAP-dependent subjects. We further tested the linkage disequilibrium in these SNPs. There was no linkage disequilibrium ($D' = 0.13$) between A118G and IVS1-A4980G in our control subjects, but disequilibrium $D' = 1.00$ corresponding to complete linkage disequilibrium in MAP-dependent subjects. IVS1-A4980G in intron 1 is located about 25 kbp downstream from exon 1. It is possible that the linkage disequilibrium block in intron 1 and exon 1 is larger than 25 kb.

We also found one nucleotide repeat downstream of IVS1-A4980G in intron 1, dinucleotide repeats IVS1-4908 $(AC)_{12-20}$. There was significant linkage disequilibrium ($P < .05$ by the Arlequin program) between IVS1-4908 $(AC)_{12-20}$ and the two SNPs tested above. A118G showed weak but significant linkage disequilibrium with IVS1-4908 $(AC)_{12-20}$ (in control subjects $D' = 0.33$, and in MAP-dependent subjects $D' = 0.46$). IVS1-A4980G showed complete linkage disequilibrium ($D' = 1.00$) with IVS1-4908 $(AC)_{12-20}$ in both control and MAP-dependent subjects. We looked for differences in the repeat polymorphism IVS1-4908 $(AC)_{12-20}$ between control and MAP-dependent subjects (TABLE 3), but no significant difference was found ($P = .83$ by CLUMP program (T1)).

We also analyzed associations of the SNPs with latency of MAP psychosis. Two groups were set up according to latency of psychosis from first MAP intake: less than three years ($n = 54$, average = 0.83 years) and more than three years ($n = 53$, average=9.98 years) (TABLE 4). The allelic frequency of SNP A118G differed sig-

TABLE 2. Allelic frequencies of SNPs in control and MAP-dependent subjects

SNP Name	Control Subjects				MAP-Dependent Subjects				
		Number	(Percent)	Allelic Frequency		Number	(Percent)	Allelic Frequency	*P* Value
A118G	A	67	(0.31)		A	50	(0.38)		
	A/G	99	(0.46)		A/G	56	(0.43)	40.5%	
	G	47	(0.22)	45.3%	G	G	(0.19)		.43
	Total	213			Total	131			
IVS1-A4980G	A	177	(0.95)		A	130	(0.94)		
	A/G	10	(0.05)		A/G	8	(0.06)	2.9%	
	G	0	(0.00)	2.7%	G	0	(0.00)		.99
	Total	187			Total	138			

TABLE 3. Allelic frequency of dinucleotide repeat in intron 1 (IVS1-4908$(AC)_{12-20}$)

Number of repeat		12	13	14	15	16	17	18	19	20	(T1)
Control	(2n = 374)	1	0	0	63	31	243	27	5	4	
	(%)	(0.3)	(0.0)	(0.0)	(16.8)	(8.3)	(65.0)	(7.2)	(1.3)	(1.1)	
MAP-dependent subjects	(2n = 276)	0	0	1	48	17	175	28	3	4	$P = .83$
	(%)	(0.0)	(0.0)	(0.4)	(17.4)	(6.2)	(63.4)	(10.1)	(1.1)	(1.4)	$x^2 = 2.70$

TABLE 4. Analysis of association between polymorphisms and latency of MAP psychosis

		Control Subjects			MAP-Dependent Subjects					
					Latency of Psychosis					
					< 3 Years			> 3 Years		
SNP Name		Number	(Percent)	Allelic Frequency	Number	(Percent)	Allelic Frequency	Number	(Percent)	Allelic Frequency
A118G	A	67	(0.31)		20	(0.38)		25	(0.46)	
	A/G	99	(0.46)	45.3%	25	(0.47)	38.7%	24	(0.44)	31.5%
	G	47	(0.22)		8	(0.15)		5	(0.09)	
	Total	213			53		$P = .47$	54		$P = .04$*
	A	177	(0.95)		48	(0.91)		51	(0.94)	
IVS1-A4980G	A/G	10	(0.05)	2.7%	5	(0.09)	4.7%	3	(0.06)	2.8%
IVS1-A4910G	G	0	(0.00)		0	(0.00)		0	(0.00)	
	Total	187			53		$P = .58$	54		$P = .75$

nificantly (chi-squared test, $P = .04$) between control subjects and MAP subjects, with latency less than three years.

Finally, we tested differences in haplotype frequencies between control and MAP-dependent subjects using genotypic data of A118G, IVS1-A4980G, and dinucleotide repeats IVS1-4908 $(AC)_{12-20}$. There was no significant difference in haplotype frequencies between the control group and either MAP group, whether latency was shorter ($P = .57$) or longer ($P = .87$).

DISCUSSION

Various evidence suggests that the μ-opioid receptor is involved in the abuse of both opiate drugs and nonopiate addictive drugs, such as alcohol, nicotine, and cocaine.[17–19] Sequence variations in both human and mouse OPRM have been reported from several groups including our own.[5–7] In humans, some SNPs (C12C, C17T, A118G, C440G, and G779A; the numbers are relative to the ATG start codon) that cause amino acid substitution (respectively, Ser4Arg, Ala6Val, Asn40Asp, Ser147Cys, and Arg260His) have already been reported.[5–7] We did not find the two SNPs (C12C, C17T) in our Japanese population. In particular, the A118G SNP in exon 1 that causes an Asn40Asp substitution is closely examined because it induces a decrease in one of the five glycosylation sites in the amino terminal of the μ-opioid receptor. *In vitro*, A118G increases the affinity of the μ-opioid receptor to one of its endogenous peptides, β-endorphin, but has no influence on any other opioid ligand.[8] Several recent studies have examined the association between frequencies of polymorphisms in OPRM (also A118G and others) and drug and/or alcohol dependence, although the results are not fully consistent.[7–14]

When MAP abusers were divided into two groups according to latency of psychosis, the allelic frequency of A118G was significantly different between the control group and MAP-dependent subjects with a latency less than three years from first MAP intake. Since we analyzed the allelic frequencies of the two SNPs in connection with MAP dependence/psychosis, Bonferroni corrections were performed on the P values. The corrected P values were $P = .08$, suggesting there is no significance.

We found linkage disequilibrium among A118G in exon 1, IVS1-A4980G, and IVS1-4908 $(AC)_{12-20}$ (from exon 1 to 25 kbp downstream) in the OPRM of our Japanese samples. Linkage disequilibrium between A118G and IVS1-A4980G was found in MAP-dependent subjects but not in control subjects. This finding suggests a correlation between MAP dependence and linkage disequilibrium. Though no significance was found in haplotype analysis, this result called for careful treatment. Hoehe *et al.*[7] identified a combination of variants, consisting mostly of a specific constellation of changes in putative transcription regulatory motifs that are found significantly more frequently in African-American substance-dependent individuals likely to have a significant genetic predisposition to their substance dependence. We did not find their sequence variants in 5′UTR in our present Japanese sample. None of the novel SNPs and dinucleotide repeats in 5′UTR and part of intron 1 in our Japanese population has been reported in African-Americans or European-Americans. Polymorphisms in the functionally relevant 5′ regulatory region of the OPRM in our Japanese population showed differences from other ethnic groups.