tion of the mixture with RNaseH and RNaseA at 37°C for 10 min. cDNA probes were purified by using a QIAquick PCR purification Kit (Qiagen Inc., Valencia, California) and quantified with a liquid scintillation counter.

To reduce the chance of producing false positives, we used three cDNA-spotted membranes per sample. Thus 30 membranes were used (3 membranes × 5 mice × 2 treatment groups). Array membranes were prehybridized for 1 h at 68°C in 0.4 mL PerfectHyb (TOYOBO Co., Osaka, Japan) hybridization buffer/membrane. Purified cDNA probes in addition to 50 μg mouse Cot-1 DNA, 2 μg polyA, and 1/100 counts Tet1 were heat-denatured (100°C for 5 min). After prehybridization, the mixture of cDNA probes were hybridized with the membranes overnight at 68°C in 10-mL vials in a rotating hybridization oven. The membranes were then washed at 68°C with 2 × SSC/1% SDS twice for 15 min each time, and at 68°C with 0.1 × SSC/1% SDS twice for 30 min each time. Thereafter they were dried and exposed to an IP plate for 2–3 days. Image acquisition and quantification were performed by using an FLA-8000 (Fuji Photo, Tokyo, Japan). Determination of the gene-expression level and the statistical treatment of the data were performed according to the calculation system provided by ArrayGage (Fuji Photo, Tokyo, Japan).

Western Blotting

Rat brains were dissected and immediately frozen until used. The tissue samples were homogenized in an ice-cold PBS buffer containing several protease inhibitors and phosphatase inhibitors. An aliquot of the homogenate was taken for protein assay, done with a BioRad protein assay kit. Samples (6–20 μg/lane) were resolved on a 5–20% gradient polyacrylamide-SDS gel, and the proteins were electrotransferred to PVDF membranes by using a semidry blotter. The membranes were then washed with TBS containing 0.05% Tween-20 (TBS/TW). Nonspecific sites were blocked with a solution consisting of 5% nonfat dry milk and 0.5% Tween-20, and then the membranes were incubated overnight at room temperature with primary antibodies (Per2, phosphorylated (p)Erk1/2, 1:5000 dilution; SCOP, 1:2500 dilution) in TBS/TW containing 10 mM NaF. The antiserum was then removed, and the membranes were washed and subsequently incubated with peroxidase-labeled secondary antibody (1:100,000 dilution; BioSource) for 1 h at room temperature. Finally, they were washed repeatedly with TBS/TW, incubated with WestDura/SuperSignal ECL reagent for 10 min, and exposed to Amersham ECL film. The intensities of the immunoreactive bands were calculated by NIHimage software, and their sizes were compared with prestained molecular-weight standards.

RESULTS

Gene Expression in Whole Brain of Mice after Chronic Methamphetamine Treatment

Using the customized cDNA microarray analysis, we found 7 out of 800 genes that had been induced at least 1.2-fold in the whole brain of mice chronically treated with methamphetamine as compared with the value for the saline-treated control animals. Only two genes, mPer2 and mKIAA0099, were significantly up-regulated (FIG. 1). However, the ratio of increase for the mKIAA0099 gene expression was

Description	*mKIAA*
Period protein homolog Per2	0347
DG-75 Murine leukemia virus	1466
Putative deubiquitinating enzyme UBPY	0055
Synaptotagmin XI	0080
REST corepressor (RCOR)	0071
ATP9A (KIAA0611) gene for ATPase	0611
unknown	1265
Ubiquitin-protein isopeptide ligase (E3)	0010
Induction by Aβ exposure	0099
Per1 interacting protein of the suprachiamatic nucleus	0443
Talin 1	0320
Peptidylprolyl isomerase A pseudogene 2	0186
unknown	0657
unknown	1388
Kinesin family member 17 (KIF 17)	1405
unknown	3010
unknown	1873

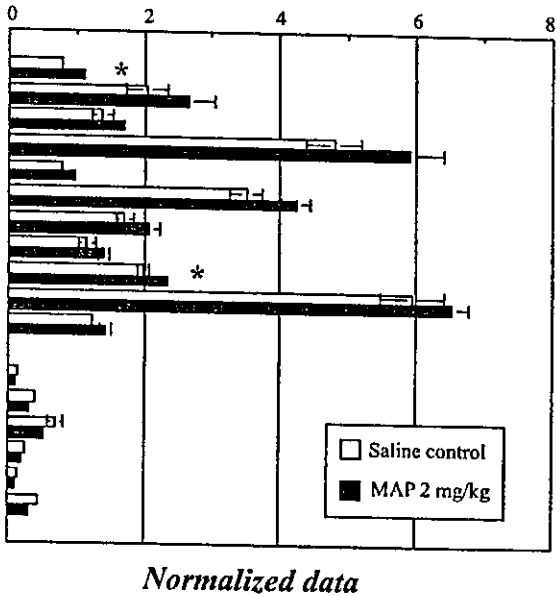

FIGURE 1. The **right panel** shows the normalized gene expression data. *Open columns* indicate the saline control; *solid gray columns* indicate the methamphetamine (MAP, 2 mg/kg, i.p.)-treated group. $N = 5$. Data were analyzed by Student's *t*-test. Statistically significant level was taken as $P < .05$. The **left panel** describes the representative mKIAA genes.

small; therefore, in the present study we focused only on the former. Per 2 is involved in circadian rhythm. Interestingly, the gene expression of Per1 interacting protein of the suprachiamatic nucleus (Pips) was slightly increased (FIG. 1). The gene expression of another period gene, SCOP (mKIAA0606), was not altered (data not shown). Other induced genes, putative deubiquitinating enzyme (UBPY), and ubiquitin-protein isopeptide ligase, are likely to be involved in the ubiquitin system. Gene expression of synaptotagmin XI, a synaptic vesicle protein, was also enhanced. However, the genes for other synaptic proteins (cysteine string protein, synaptophysin, vesicle-associated calmodulin-binding protein) were not affected (data not shown). On the other hand, six genes were reduced less than 0.8-fold by the chronic methamphetamine treatment (FIG. 1). To investigate more precisely the role of period genes in drug abuse, we assessed changes in the expression of Per2 and related genes in various brain regions.

Effects of Chronic Amphetamine Treatment on the Expression of Period-Related Genes in Rat Brain

Immunoblot analysis with anti-Per2 antibody or anti-SCOP antibody revealed a single band of ~100 kDa (Per2) or ~180 kDa (SCOP), as shown in FIGURE 2. Chronic treatment with the lower concentration of methamphetamine (4 mg/kg) did not alter the level of Per2 immunoreactivity (IR) in any brain areas. However, that with the higher concentration (8 mg/kg) significantly increased the Per2 IR level in the hippocampus, whereas it moderately decreased the level in the hypothalamus (FIG. 2), and slightly decreased it in the midbrain, cortex (not including the frontal cortex), and cerebellum (data not shown). In contrast, the lower concentration of methamphetamine treatment increased the SCOP IR level in the hippocampus (FIG. 2). However, the higher concentration (8 mg/kg) did not induce any significant change in SCOP IR in any brain area. Since acute administration of amphetamine was reported to cause an increase in the level of pErk1/2 IR,[14] we examined the effect of chronic treatment with methamphetamine on pErk1/2 IR. However, the chronic treatment with methamphetamine had no significant effect on the pErk1/2 IR level in any brain region examined.

DISCUSSION

The study presented here used a customized cDNA microarray to find candidate genes affected by chronic methamphetamine treatment. The beneficial aspects of using this customized microarray are that mouse homologues of the KIAA cDNA are plotted on membranes. Because this was our first use of this microarray, we carefully designed the experiment. It already had been reported that higher doses of methamphetamine treatment caused significant changes in genes related to cellular stress chaperones and protein degradation.[6,10] We therefore at first chose a low dose of methamphetamine to eliminate genes related to toxicity, such as those of heat-shock proteins. Next, to obtain reliable data, we made multiple biological repeats, that is, mRNA extractions from independent mouse tissues and multiple membrane replicates, that is, one sample was measured by using three cDNA membranes. These procedures were necessary for statistical analysis and for the generalization of con-

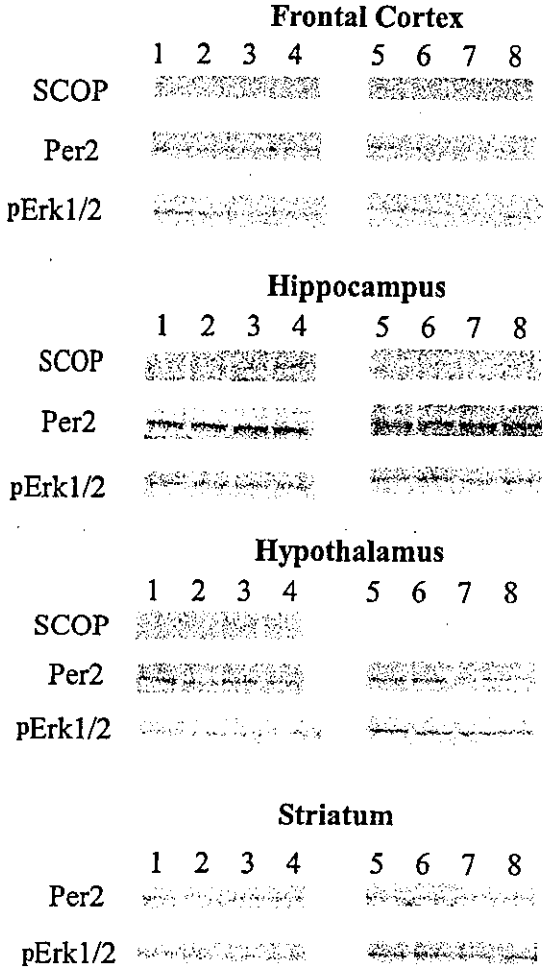

FIGURE 2. Western immunoblot analysis of the effect of chronic treatment with saline or methamphetamine for 10 days on SCOP, Per2, and pErk1/2 IR in the indicated brain regions. A dissected brain region from each rat was subjected to SDS-PAGE; then immunoblot analysis was done by using antibodies against SCOP, Per2, and p Erk1/2. *Lanes 1, 2, 5, and 6* correspond to the saline control. *Lanes 3 and 4* correspond to rat treated with 4 mg/kg of methamphetamine. *Lanes 7 and 8* correspond to rat treated with 8 mg/kg of methamphetamine. Semiquantitative analysis showed that methamphetamine (4 mg/kg, i.p.) increased the SCOP IR in the hippocampus (2.17-fold, $n = 7$; $P < .05$ vs. saline control), and that methamphetamine (8 mg/kg, i.p.) increased the Per2 IR in the hippocampus (1.58-fold, $n = 5$; $P < .05$ vs. saline control).

clusions. Finally, to confirm the obtained data, we performed Western blot analysis and verified the relative changes in the expression.

mPer2 was the major candidate gene with altered expression after repeated methamphetamine treatment, as judged from the cDNA microarray results. Per genes are involved in a negative transcriptional feedback loop in the suprachiasmatic nucleus (SCN).[15] Although SCN is a well-known master pacemaker, expression of Per genes is not restricted to the SCN; however, the functions of these genes outside the SCN are not clear. Using immunoblot analysis, we found a significant increase in the expression level of Per2 protein in the hippocampus at a dose of 8 mg/kg methamphetamine. In the case of repeated cocaine exposure, enhanced drug craving was eliminated in flies with mutations for period, clock, cycle, and double-time.[16] In a study of knockout mice, mPer2 mutant mice exhibited a hypersensitivity to cocaine,[17] thus suggesting the possibility that deletion or reduction of Per2 may enhance the addictive response to cocaine.

On the other hand, SCOP is predominantly expressed in the brain that was expressed in a circadian manner in the SCN;[18] its physiological function is not clear. Using the cDNA microarray, we found that SCOP gene expression in the whole brain was not affected by the chronic methamphetamine treatment. However, the hippocampal expression was increased by the repeated methamphetamine administration (4 mg/kg). Shimizu *et al.*[19] reported that SCOP associated with the nucleotide-free form of K-Ras in membrane rafts to down-regulate the Ras-mitogen–activated pro-

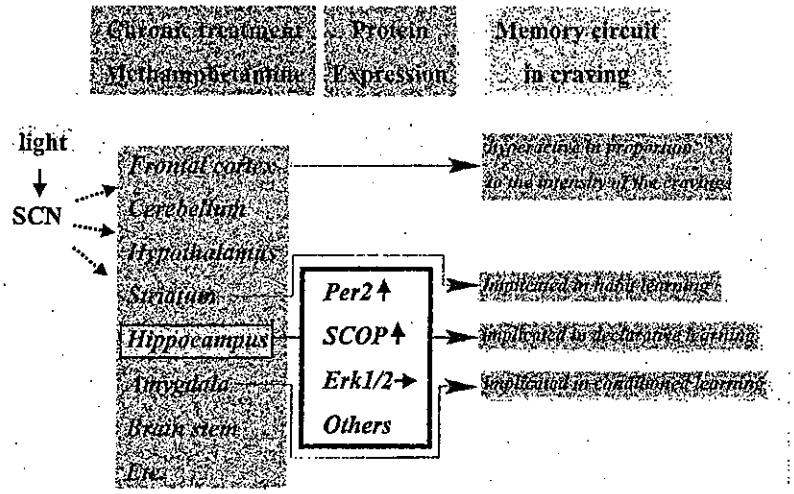

FIGURE 3. Methamphetamine abuse and circadian rhythm. Craving is a very difficult problem when it comes to terminating the self-administration of drugs of abuse. In our study, Per2 and SCOP expressions in the hippocampus were affected after chronic methamphetamine treatment. Because the Per gene is involved in the stength of craving,[7] the next step is to discover the relationship between the expression of this gene in the hippocampus and memory, especially declarative learning.

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.

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

Focus on the Mu Opioid Receptor Gene

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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

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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.

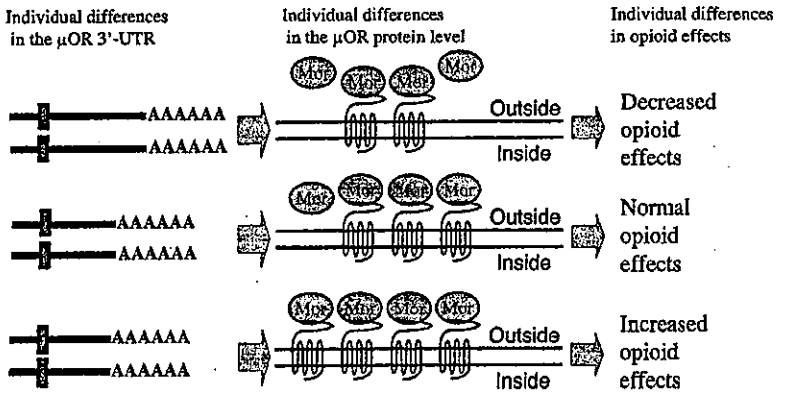

FIGURE 1. Schematic illustration indicating a possible genetic mechanism underlying individual differences in opioid effects. Variation in the 3′-UTR of muOR may cause differences in muOR protein expression and, in turn, result in the reduced opioid responses. Mor, morphine.

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.

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

Potentially Therapeutic Agents for Addictive Drug Users

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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**

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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,

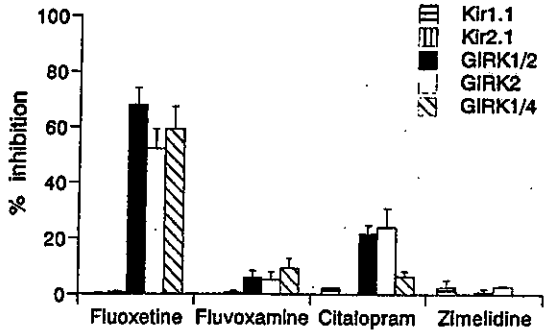

FIGURE 1. Comparison of the SSRI effects on members of inwardly rectifying potassium channels: Kir1.1, Kir2.1, and GIRK channels, expressed as homomeric or heteromeric channels in *Xenopus* oocytes. The concentration of the SSRIs used was 100 μM. Current responses were measured at a membrane potential 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.

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Gene Polymorphisms of the Mu Opioid Receptor in Methamphetamine Abusers

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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-**

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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