Single-nucleotide polymorphism in the human mu opioid receptor gene alters β-endorphin binding and activity: Possible implications for opiate addiction

CHERIE BOND*, K. STEVEN LAFORGET†, MINGTING TIAN*, DOROTHY MELIA†, SHENGWEN ZHANG*‡, LISA BORG†, JIANHUA GONG*‡, JAMES SCHLUGER†, JUDITH A. STRONG*‡, SUZANNE M. LEAL§, JAY A. TISCHFIELD*, MARY JEANNE KREEK†, AND LEI YU*‡¶

*Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202-5251; ‡Department of Cell Biology, Neurobiology and Anatomy, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521; and §Laboratory of Statistical Genetics, The Rockefeller University, New York, NY 10021-6399

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ABSTRACT Opioid drugs play important roles in the clinical management of pain, as well as in the development and treatment of drug abuse. The mu opioid receptor is the primary site of action for the most commonly used opioids, including morphine, heroin, fentanyl, and methadone. By sequencing DNA from 113 former heroin addicts in methadone maintenance and 39 individuals with no history of drug or alcohol abuse or dependence, we have identified five different single-nucleotide polymorphisms (SNPs) in the coding region of the mu opioid receptor gene. The most prevalent SNP is a nucleotide substitution at position 118 (A118G), predicting an amino acid change at a putative N-glycosylation site. This SNP displays an allelic frequency of approximately 10% in our study population. Significant differences in allele distribution were observed among ethnic groups studied. The variant receptor resulting from the A118G SNP did not show altered binding affinities for most opioid peptides and alkaloids tested. However, the A118G variant receptor binds β-endorphin, an endogenous opioid that activates the mu opioid receptor, approximately three times more tightly than the most common allelic form of the receptor. Furthermore, β-endorphin is approximately three times more potent at the A118G variant receptor than at the most common allelic form in agonist-induced activation of G protein-coupled potassium channels. These results show that SNPs in the mu opioid receptor gene can alter binding and signal transduction in the resulting receptor and may have implications for normal physiology, therapeutics, and vulnerability to develop or protection from diverse diseases including the addictive diseases.

The mu opioid receptor is the primary site of action of several of the endogenous opioid peptides including β-endorphin, Met-enkephalin-Arg-Phe, and the recently identified endorphins (1). This receptor is also the major target for clinically important opioid analgesic agents including morphine, methadone, fentanyl, and related drugs (2, 3). Activation of this receptor has diverse physiological effects (4, 5). Furthermore, it is the major molecular site of action for heroin (6, 7). Rapid activation of the mu opioid receptor, such as that which occurs in the setting of drug abuse, results in a euphoric effect, thus conferring the reinforcing or rewarding effects of the drug, contributing to the development of addiction. Clinical observations have suggested that individuals have varied sensitivity to opioids, suggesting potential variability in the receptor protein and gene.

Naturally occurring polymorphisms are well known to exist in human genes; some have been shown to produce profound effects on the function of the corresponding proteins. Molecular cloning of the mu opioid receptor (8–11) has made it possible to determine potential sequence polymorphism, as shown by two recent studies (12, 13). The mu opioid receptor is a member of the G protein-coupled receptor family (8, 14). There are a number of well documented cases where naturally occurring mutations in G protein-coupled receptors lead to malfunctioning proteins and disorders in humans. Examples include a single-nucleotide polymorphism (SNP) in the luteinizing hormone (LH) receptor gene that results in a constitutively activated LH receptor at the cellular level and the development of precocious puberty in young male children (15); SNPs in the thyrotropin receptor gene that cause constitutive activation of adenyl cyclase and hyperfunctioning thyroid adenomas (16); and mutations in the vasopressin receptor gene that cause truncation of the receptor and alter its function (17).

To further identify SNPs in the mu opioid receptor and to characterize whether receptor activity may be altered by SNPs, we studied DNA samples from subjects including both former heroin addicts in methadone maintenance treatment and individuals with no history of opiate or nonopiate drug or alcohol dependence. Here we report the findings that, indeed, SNP polymorphism in the mu opioid receptor gene can affect the activity of the mu opioid receptor, changing its sensitivity to the endogenous agonist β-endorphin.

MATERIALS AND METHODS

Study Subjects and Procedures. Addictive disease patients, who were long-term former heroin addicts currently in chronic methadone maintenance treatment, and also normal control subjects with no history of any drug or alcohol abuse, were included in this study. All study subjects were characterized extensively with respect to drug abuse, the addictive diseases, psychological and psychiatric profiles, and medical and ethnic family backgrounds. No study subjects were related.

Previously heroin-addicted patients admitted to the study conformed to the federally regulated criteria for admission to a methadone or L-alpha-acetyl-methadone (LAAM) maintenance program. These criteria are: (i) 1 or more years of daily multiple-dose self-administration of heroin or other opiates, (ii) the development of tolerance and dependence, and (iii) drug-seeking behavior (18). All opioid-dependent study sub-

Abbreviation: SNP, single-nucleotide polymorphism.

*To whom reprint requests should be addressed at: Department of Cell Biology, Neurobiology and Anatomy, University of Cincinnati College of Medicine, 231 Bethesda Avenue, MSB-G163, Cincinnati, OH 45267-0521. e-mail: lei.yu@uc.edu.

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jects were former heroin addicts currently in treatment at methadone maintenance clinics in New York City, primarily, two clinics closely associated for more than 25 years with the Laboratory of the Biology of Addictive Diseases at The Rockefeller University, the Adolescent Development Program and Adult Clinic at the New York–Cornell Medical Center. Current or prior abuse of other drugs was not used as an exclusion criterion for this study group as long as long-term opiate addiction continued to be the primary diagnosis.

Healthy control subjects were recruited primarily through posting of notices and newspaper advertisements or by referral as possible research volunteers by physicians or staff at The Rockefeller University Hospital. Individuals with any ongoing drug or alcohol abuse, or prior periods of alcohol or drug abuse, were excluded from this category. The exclusion criteria were defined as follows: for current or continuing abuse of alcohol, at least one instance of drinking to intoxication during the previous 30 days; for opiates, cocaine, amphetamines, or other illicit drugs (excluding cannabis), any use during the previous 30 days. Users of nicotine or caffeine were not excluded, nor were individuals who had abused cannabis for up to 12 days during the previous 30 days. For prior abuse, subjects were excluded who had used illicit drugs (with the exception of cannabis) for at least three times a week for a period of at least 1 month at any point in their lifetime.

All study subjects were screened rigorously by specially trained research personnel, including psychiatrists and research nurses, to ensure appropriate characterization of addictive diseases, status of treatment, and presence or absence of any polydrug or alcohol abuse. Both addictive disease patients and normal volunteers admitted to the study were assessed by a psychiatrist or research nurse with several general and specific psychiatric and psychological instruments. The Addiction Severity Index was included, with previously established interobserver reliability for the trained clinical research staff involved in this study (19). Study subjects also were administered a detailed personal, medical, and specific addictive disease questionnaire. Urine specimens were obtained on all study subjects, both those in the addictive disease group (in whom regular, ongoing urine collections are made and analyzed both for clinical care and other research purposes), and in the healthy, normal volunteer group. Urine specimens were analyzed for multiple drugs of abuse. Although not utilized in the present study, a family medical history and addictive disease questionnaire, designed to provide information regarding any substance abuse and major mental illnesses of first- and second-degree relatives, was administered.

Study subjects provided detailed information regarding family origin and ethnic background, including country or geographic area of birth. This information was obtained for both the study subjects and their immediate ancestors (parents, grandparents, and great-grandparents) to the extent that the information was known by the study subjects. Study subjects were classified into five groups: African-American, Caucasian, Hispanic (Caribbean and Central or South American origin), Native North American, and Other.

Subjects entering the study were required to be competent to understand the study procedures and understand and sign the Institutional Review Board-approved informed consent. Patients with schizophrenia or other psychotic mental illnesses were excluded from the study by this criterion. The presence of any known serological markers for hepatitis B or C or HIV was not used as an exclusion criterion.

After the informed consent, psychiatric and behavioral assessment, and family history acquisition, venipuncture on any known serological markers for hepatitis B or C or HIV of any known serological markers for hepatitis B or C or HIV was performed. Although not utilized in the present study because this exon is small (4 or 12 aa residues) and alternative splicing in this exon has been shown to occur (20). Exon 1 forward primer sequences were based on the 5' untranslated region of the receptor (11). Exon 1 reverse, exon 2 forward and reverse, and exon 3 forward primer sequences were based on partial intron sequence data obtained from inverse PCR of genomic DNA sequences for the receptor gene (data not shown). Exon 3 reverse primers were based on reported intron 3 sequence (20). Two sets of primers were designed for each exon to allow for nested PCR to increase amplification specificity of the exonic regions. Only one reverse primer was used for exon 1. The PCRs were performed with 300–400 ng of genomic DNA, PCR products were separated on agarose gels, and the DNA fragments were purified for DNA sequencing. DNA polymorphisms were confirmed by both manual and automated sequencing.

**Mutagenesis.** In vitro site-directed mutagenesis was performed to generate human mu opioid receptor (hMOR) cDNA containing one of two major variants found, the A118G SNP. Complementary oligonucleotides containing the desired mutation were synthesized and annealed to the pcDNA3 plasmid containing the most common allele of hMOR. Primer 1, TTGTCCCACTTAGATGGCGACCTGTC- and primer 2, ACCGCATGGTGCCAGAGGTCGCCATCTAAAGTG. Primers were extended and the product was amplified by PCR using hMOR dsDNA as the template, and DpnI restriction enzyme was added afterward to digest the methylated, nonmutated, most common dsDNA. After transformation into Escherichia coli cells, DNA from individual colonies was examined by restriction enzyme digestion and DNA sequencing to confirm success of mutagenesis.

**Cell Transfection and Binding Analysis.** Stable transfection of the A118G SNP plasmid into AV-12 cells was performed (21). Individual colony DNA from each plasmid was harvested by washing with PBS at room temperature, scraped into homogenization solution (0.3 M sucrose/25 mM Tris-HCl, pH 7.4/0.05% BSA and protease inhibitor cocktail, including 0.5 mM phenethylmethylsulfonyl fluoride/0.1 μg/ml leupeptin/0.01% aprotinin), transferred to Dounce homogenizer, and homogenized on ice. The suspension was centrifuged at 1,000 × g for 10 min, and the supernatant was saved in a clean tube. The cell pellet was resuspended in homogenization buffer, homogenized, and centrifuged as described above. The supernatants from both extractions were combined and centrifuged at 30,000 × g for 20 min. The pelleted membranes were resuspended in binding buffer (50 mM Tris-HCl, pH 7.4); binding assays were carried out using membrane protein preparations as described (11). It has been shown that N-linked glycosylation is carried out by AV-12 cells (22, 23).

**Electrophysiology.** Preparation of Xenopus oocytes was as reported previously (11). Oocytes were injected with in vitro transcribed mRNAs for the most common or A118G variant mu opioid receptors together with the G protein-activated, inwardly rectifying K+ channels (GIRK1 and GIRK2). Two to 3 days after RNA injection, oocytes were voltage-clamped in ND96 solution (96 mM NaCl/2 mM KCl/1 mM MgCl2/1.8 mM CaCl2/5 mM Hepes, pH 7.6) by using a two-electrode...
oocytes have been shown to carry out N-linked glycosylation
ligands to measure the resulting potassium current.

Ethnicity

mM CaCl2

*The two individuals who were not classified into African-American, Caucasian, or Hispanic ethnic groups were not included in the analysis.

Opioid Dependence

83 males (56.4%).

alcohol abuse. Study subjects included 69 females (45.4%) and
dependence or any ongoing illicit opiate or other drug use, or
study subjects (25.7%) had no history of drug or alcohol
previous or current codependency for other substances; 39
thirteen of the study subjects (74.3%) were former heroin
section were met by these 152 individuals. One hundred and
DNA samples from 152 subjects, including both former heroin
amplified exonic regions. Using this method, we sequenced
it possible to determine potential sequence polymorphism. To

RESULTS AND DISCUSSION

Polymorphisms in the Human mu Opioid Receptor Gene.

Molecular cloning of the mu opioid receptor (8–11) has made
it possible to determine potential sequence polymorphism. To
identify SNPs in the mu opioid receptor, we used a PCR-based
strategy to amplify the coding regions of the mu opioid
receptor gene and to determine the DNA sequence of the
amplified exonic regions. Using this method, we sequenced
DNA samples from 152 subjects, including both former heroin
addicts in methadone maintenance treatment and individuals
with no history of drug or alcohol dependence.

Inclusion criteria as defined in the Materials and Methods
section were met by these 152 individuals. One hundred and
thirteen of the study subjects (74.3%) were former heroin
addicts in methadone maintenance treatment, with or without
previous or current codependency for other substances; 39
study subjects (25.7%) had no history of drug or alcohol
dependency or any ongoing illicit opiate or other drug use, or
alcohol abuse. Study subjects included 69 females (45.4%) and
83 males (56.4%).

Within the group of former heroin addicts in methadone
maintenance treatment, the mean years in treatment was 6.7
with a range from 2 months to 30 years (n = 112, only one
patient’s history could not be verified by an additional inter-
view or by medical chart records). Before treatment, the mean
years of heroin addiction was 10.1 years, with a range from 1
to 30 years (n = 109; the exact duration of heroin use before
entry into methadone treatment could not be verified for four
subjects). The mean daily methadone dose of opioid depend-
ent patients in stable treatment was 84 mg/day, with a range
from 30 to 120 mg/day. Only patients (n = 106) with estab-
lished stable methadone doses were included in this calcula-
tion; the other patients were undergoing induction, increasing,
tapering, or elimination dose schedules.

The ethnic breakdown of the study subject populations was
as follows: African-American, 31 (20.3%); Caucasian, 52
(34.2%), Hispanic, 67 (44.1%), Native North American, 1
(0.7%), and Other, 1 (0.7%). Several individuals reported that
one parent’s ancestry was from one ethnic group and the other
parent’s ancestry was from a second ethnic group, including
four individuals (2.6%) who reported one African-American
parent and one Caucasian parent, and five individuals (3.3%)
who reported one Caucasian parent and one Hispanic. For the
genotype calculations, the former study subjects were classified
as African-American and the latter, as Hispanic.

By sequencing PCR-amplified DNA from the study subjects,
we determined that the previously reported sequence for the
human mu opioid receptor (10, 11) was the most common
allele found in our study population. We also identified five

Table 1. SNPs in the human mu opioid receptor gene

<table>
<thead>
<tr>
<th>Variant name</th>
<th>Nucleotide position</th>
<th>Exon location</th>
<th>Corresponding amino acid change</th>
<th>Protein domain</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A118G</td>
<td>118</td>
<td>1</td>
<td>Asn-40 → Asp (N40D)</td>
<td>N terminal</td>
<td>10.5% (26 heterozygous individual and 3 homozygous individuals, n = 152)</td>
</tr>
<tr>
<td>C17T</td>
<td>17</td>
<td>1</td>
<td>Ala-6 → Val (A6V)</td>
<td>N terminal</td>
<td>6.6% (14 heterozygous individuals and 3 homozygous individuals, n = 152)</td>
</tr>
<tr>
<td>G24A</td>
<td>24</td>
<td>1</td>
<td>Silent mutation</td>
<td>N terminal</td>
<td>2% (six heterozygous individuals, n = 152)</td>
</tr>
<tr>
<td>G779A</td>
<td>779</td>
<td>3</td>
<td>Arg-260 → His (R260H)</td>
<td>CL3</td>
<td>One heterozygous individual</td>
</tr>
<tr>
<td>G942A</td>
<td>942</td>
<td>3</td>
<td>Silent mutation</td>
<td>EL3</td>
<td>One heterozygous individual</td>
</tr>
</tbody>
</table>

Nucleotide position 1 is the first base of the start codon. Protein domains are based on the seven-transmembrane model for opioid receptors. EL, extracellular loop; CL, cytoplasmic loop.

Table 2. Genotype and allele frequency associations

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>A118G Genotypes</th>
<th>A118G Alleles</th>
<th>C17T Genotypes</th>
<th>C17T Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/A A/G G/G Total</td>
<td>A G Total</td>
<td>C/C C/T T/T Total</td>
<td>C T Total</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>30 (0.968) 1 (0.032) 0</td>
<td>31 (0.984) 1 (0.016) 62 (0.677) 7 (0.072) 3 (0.047) 31 (0.790) 22 (0.149) 62 (0.677) 20 (0.127) 6 (0.037) 31 (0.790) 13 (0.088) 62 (0.677) 20 (0.127) 6 (0.037)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>41 (0.788) 10 (0.192) 1</td>
<td>52 (0.885) 12 (0.215) 104 (1.000) 2 (0.019) 104 (1.000) 2 (0.019) 104 (1.000) 2 (0.019) 104 (1.000) 2 (0.019)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>50 (0.746) 15 (0.225) 2</td>
<td>67 (0.858) 19 (0.286) 134 (1.340) 7 (0.035) 67 (0.858) 19 (0.286) 134 (1.340) 7 (0.035) 67 (0.858) 19 (0.286) 134 (1.340) 7 (0.035)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>59 (0.855) 8 (0.116) 2</td>
<td>69 (0.913) 12 (0.187) 138 (1.380) 8 (0.116) 69 (0.913) 12 (0.187) 138 (1.380) 8 (0.116) 69 (0.913) 12 (0.187) 138 (1.380) 8 (0.116)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64 (0.771) 18 (0.217) 1</td>
<td>83 (0.880) 20 (0.120) 166 (1.660) 5 (0.060) 83 (0.880) 20 (0.120) 166 (1.660) 5 (0.060) 83 (0.880) 20 (0.120) 166 (1.660) 5 (0.060)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opioid Dependence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dependent</td>
<td>94 (0.832) 18 (0.159) 1</td>
<td>113 (0.912) 20 (0.188) 226 (2.260) 7 (0.075) 113 (0.912) 20 (0.188) 226 (2.260) 7 (0.075) 113 (0.912) 20 (0.188) 226 (2.260) 7 (0.075)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondependent</td>
<td>29 (0.744) 8 (0.205) 2</td>
<td>39 (0.846) 12 (0.154) 78 (0.780) 1 (0.013) 39 (0.846) 12 (0.154) 78 (0.780) 1 (0.013) 39 (0.846) 12 (0.154) 78 (0.780) 1 (0.013)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The two individuals who were not classified into African-American, Caucasian, or Hispanic ethnic groups were not included in the analysis.
different variants, all SNPs. For the purpose of this report, we use the term “most common” to denote the predominant mu opioid receptor allele and the corresponding receptor that was reported originally by cDNA cloning (10, 11), and the term “variant” to denote the allelic genes or the resulting receptors containing polymorphic variations. Table 1 shows these SNPs, with information on the position of amino acid substitutions, and overall frequency of the variant alleles in the study population. Two recent studies (12, 13) also have identified one or both of these five SNPs in the coding region (A118G and C17T), but not the other three SNPs (G24A, G779A, and G942A).

Genotype and allele frequencies for the two most prevalent allelic variants in our population sample, the A118G and C17T polymorphisms, are shown in Table 2, with overall allelic frequencies of 10.5 and 6.6%, respectively. One of the two earlier reports, comparing healthy volunteers with cocaine-and/or opiate-dependent individuals, found an allelic frequency of 10 and 22% for the C17T receptor SNP in their two study groups, respectively (12). This study included Caucasian and African-American subjects. The A118G SNP was not identified (12). The second previously reported study included American Caucasian, Finnish Caucasian, and Native North American study subjects; an allelic frequency of 10.5–16.3% and overall frequency of the variant alleles in the study population. The test for heterogeneity of the Relative Risk among ethnic groups was not significant \[\chi^2(1) = 8.22 \ (P = 0.0041)\]. Although this finding could be explained by population admixture within this group, it suggests the possibility that the A118G SNP might confer a relative protection against opioid dependence. Further studies with a larger sample size would be necessary to test this hypothesis.

In contrast to the A118G allele, the C17T variant was present in a higher overall proportion of opioid-dependent persons in our sample at a marginal significance level \[\chi^2(1) = 3.70 \ (P = 0.054)\]. This result is similar to that obtained by Berrettini et al. (12).

Table 3 shows data of this study stratified by ethnic group and opioid-dependency status for both the A118G and the C17T SNPs. The pooled Relative Risk (RR) and the Mantel–Haenszel Chi-square \(26\) were calculated. For the A118G polymorphism there was no significant difference in allele frequencies between former heroin addicts in treatment (cases) and those with no history of drug or alcohol abuse or dependence (controls) \[RR = 0.48 \ x^2(5) = 2.76 \ (P = 0.096)\]. Although not significant, with respect to the Relative Risk there was evidence of heterogeneity among ethnic groups \[x^2(2) = 5.16 \ (P = 0.076)\]. It should be noted that the direction of the Relative Risk, i.e., less than one, shows here that the A118G polymorphism was more frequent in control subjects than in opioid-dependent subjects and, again, suggests that the A118G polymorphism might confer some level of protection against opiate addiction, which is of particular interest given the differences in receptor activity (see below). There was a marginally significant difference in the allele frequencies for the C17T polymorphism between opioid-dependent and healthy control subjects \[RR = 7.83 \ x^2(5) = 3.73 \ (P = 0.05)\]. The test for heterogeneity of the Relative Risk among ethnic groups for the C17T polymorphism was not significant \[x^2(2) = 3.95 \ (P = 0.14)\].

The A118G SNP Affects β-Endorphin-Binding Affinity of the mu Opioid Receptor. The most prevalent genetic polymorphism we identified is the A118G SNP with a substitution at the nucleotide position 118 with respect to the first base of the

Table 3. Stratification of alleles of opioid-dependent and nondependent study subjects by ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>A118G Allele Freq.</th>
<th>C17T Allele Freq.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Opiodependent)</td>
<td>(Nondependent)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>African-American</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Hispanic</td>
<td>104</td>
<td>12</td>
</tr>
<tr>
<td>Combined</td>
<td>202</td>
<td>20</td>
</tr>
</tbody>
</table>

The two individuals who were not classified as African-American, Caucasian, or Hispanic ethnic groups were not included in the analysis.
We performed radioligand-binding assays with cell lines stably transfected with either the A118G variant or the most common mu receptor to determine whether the A118G polymorphism changes the receptor’s ability to bind opioid ligands, especially endogenous opioid peptides, because they are the physiological agonists for the mu opioid receptor. The A118G variant and the most common mu receptors yielded similar binding affinity values for most of the opioid ligands tested, including the small endogenous peptide agonists Met- and Leu-enkephalin, each with five aa residues; endomorphin-1 and -2, each with four residues; the mu-selective synthetic opioid peptide DAMGO, with five amino acid residues; the endogenous ligand for the kappa opioid receptor dynorphin A (1–17); as well as the mu-prefering opioid alkaloid agonists morphine, fentanyl, methadone, and the opioid antagonist naloxone (Fig. 2, and data not shown). These results suggest that the A118G polymorphism does not change the overall binding properties of the mu opioid receptor. This is not unexpected, because the predicted amino acid change as a result of the A118G SNP is a single residue substitution in the N-terminal region in the extracellular space and is unlikely to drastically affect the overall tertiary structure of the receptor.

There was a substantial change for the A118G variant receptor binding of human β-endorphin, a much larger endogenous opioid peptide, which has 31 aa residues and which activates the mu opioid receptor. Whereas the other, smaller, endogenous opioid peptides and alkaloid agonists and antagonist displayed similar binding affinities for both receptors, the A118G variant receptor showed higher binding affinity for β-endorphin than the most common receptor (Fig. 2), with the ratio of the Kᵢ of the most common allele to A118G variant SNP being 3.46 ± 0.31 (mean ± SEM, n = 3). These results indicate that although the A118G polymorphism did not alter the overall profile of ligand binding to the receptor, it specifically influenced the β-endorphin binding and resulted in much higher affinity for this large, endogenous opioid peptide.

We found that the β-endorphin binds significantly more strongly to the A118G variant receptor than the most common receptor; therefore, it is likely that the A118G polymorphism changes the receptor’s ability to bind this class of ligands in vivo. We have previously reported that the A118G SNP does not alter the overall profile of ligand binding to the most common mu opioid receptor, and that the A118G polymorphism changes the receptor’s ability to bind opioid ligands, especially endogenous opioid peptides, because they are the physiological agonists for the mu opioid receptor. This is not unexpected, because the predicted amino acid change as a result of the A118G SNP is a single residue substitution in the N-terminal region in the extracellular space and is unlikely to drastically affect the overall tertiary structure of the receptor.

**Fig. 3.** Comparison of the most common and the A118G variant mu opioid receptors in coupling to G protein-activated, inwardly rectifying potassium channels (GIRK) channels (31, 32). Coexpression studies have shown that the mu opioid receptor can readily activate GIRK channels via a G protein-mediated mechanism (11, 33, 34). To examine the effect of the A118G polymorphism, we used the *Xenopus* oocyte expression to compare the A118G variant receptor with the most common mu opioid
receptor. Agonist stimulation of the A118G variant receptor activated a potassium current similar to that seen with the most common mu opioid receptor (11, 33). The EC50 values for endorphin-1 are 4.6 nM for the most common receptor and 4.9 nM for the A118G variant receptor (Fig. 3), indicating that endorphin-1 activated both receptors with similar potency. The EC50 values for β-endorphin, however, differed about 3-fold between the A118G variant and the most common mu opioid receptors (Fig. 3), consistent with the change in the binding affinity (Fig. 2). These data indicate that, as a result of the SNP in the receptor gene, the A118G variant receptor may be functionally different from the most common mu opioid receptor.

An endogenous opioid with wide distribution in both the central nervous system (CNS) and the periphery, β-endorphin has been postulated to play a role in diverse biological functions (35, 36). As a neuropeptide, it can modulate neurotransmitter actions in the CNS to mediate antinociception. It is also a mediator of the stress response, of potential importance for the pathophysiology of the addictive diseases (37–42). β-endorphin can regulate the secretion of both stress and reproductive hormones, thereby influencing a variety of physiological functions. Given the diverse roles of β-endorphin, it is particularly interesting that the A118G polymorphism may change the receptor with respect to the binding affinity of β-endorphin and the potency of its cellular activity. The approximately 3-fold difference in binding affinity and potency of β-endorphin (Figs. 2 and 3) suggests that individuals carrying the variant receptor gene (A118G) may show differences in some of the functions mediated by β-endorphin action at the altered mu opioid receptors. Thus, for example, response to stress, reproductive function, and pain perception could be altered. Moreover, the data suggest that subtle changes, such as the SNP studied with respect to its binding and activity, could have significant effects on the susceptibility or vulnerability to develop multifactorial diseases such as specific addictions (7, 8, 43).

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