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Identification of Four Novel Exon 5 Splice Variants of the Mouse μ-Opioid Receptor Gene: Functional Consequences of C-Terminal Splicing

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ABSTRACT

The rat μ-opioid receptor clone in which novel exon 5 was found in the place of exon 4 (MOR-1B) was one of the first MOR-1 variants described. We now have identified the mouse homolog of the rat MOR-1B as well as four additional variants derived from splicing from exon 3 into different sites within exon 5. The sequences of all of the variants were identical except for the intracellular tip of the C terminus encoded by exon 5, where each variant predicted a unique amino acid sequence ranging from 2 to 39 amino acids. All of the mMOR-1B variants were selective for μ-opioids in receptor-binding assays, as anticipated, because they all have identical binding pockets defined by the transmembrane domains. However, the relative potency and efficacy of μ-agonists to each other varied from variant to variant in guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate-binding studies, as shown by morphine-6β-glucuronide, which was the most efficacious agent against mouse MOR-1B1 (mMOR-1B1) and the least efficacious agent against mMOR-1B2. mMOR-1B4 was quite unusual. Although mMOR-1B4 was μ-selective in receptor-binding studies and antagonists labeled mMOR-1B4 well, the binding affinities of most of the μ-agonists were far lower than those seen with mMOR-1, suggesting that the 39 amino acids at the C terminus of mMOR-1B4 influences the conformation of the receptor and its ligand recognition site itself either directly or through its interactions with other proteins. In conclusion, alterations in the amino acid sequence of the C terminus do not alter the μ-specificity of the receptor but they can influence the binding characteristics, efficacy, and potency of μ-opioids.

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After the classification of the μ-, κ-, and δ-opioid receptors (Martin et al., 1976; Lord et al., 1977; Kosterlitz and Leslie, 1978), drugs were classified by their selectivity profiles in receptor-binding assays. Most clinical analgesics are μ-selective, having little affinity for either κ- or δ-receptors, yet clinicians have long known that patient responses to μ-opioids can vary widely, both in terms of their relative analgesic activity and their side effects (Payne and Pasternak, 1992). Clinicians also have used incomplete cross-tolerance among μ-opioids to regain analgesic activity in patients highly tolerant to one μ-drug by switching them to a different μ-opioid, a technique termed opioid rotation (Cherny et al., 2001). These and other clinical observations raised the possibility that all of the μ-opioids might not be acting through identical receptor mechanisms.

The concept of multiple μ-opioid receptors was formally proposed almost 25 years ago (Wolozin and Pasternak, 1981) based on identification of a novel receptor-binding site (Pasternak and Snyder, 1975a; Lutz et al., 1984) and the actions of the novel antagonists naloxonazone and naloxonazine, agents that dissociated opioid analgesia from other opioid actions, including respiratory depression, the inhibition of gastrointestinal transit, and most signs of physical dependence (Pasternak et al., 1980; Ling et al., 1984, 1985; Heyman et al., 1988).

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ABBREVIATIONS: MOR-1, μ-opioid receptor clone-1; mMOR-1, mouse MOR-1; rMOR-1, rat MOR-1; ANOVA, analysis of variance; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; nor-BNI, norbinaltorphimine; [Dmt^1]DALDA, Dmt-D-Arg-Phe-Lys-NH_2; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2; M6G, morphine-6β-glucuronide; U50,488H, *trans*-(*d*,*l*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide; Gpp(NH)p, guanosine-5′-[(β,γ)-imido]triphosphate; GTPγS, guanosine 5′-*O*-(thiotriphosphate); DADLE, [D-Ala^2,D-Leu^5]-enkephalin; CHO, Chinese hamster ovary; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s).

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The cloning of the μ-opioid receptor MOR-1 opened the study of μ-opioid receptors at the molecular level (Chen et al., 1993; Eppler et al., 1993; Thompson et al., 1993; Wang et al., 1993). Soon after the initial reports, two MOR-1 splice variants were identified, providing the first appreciation of the complexity of the μ-opioid receptor gene, *Oprm* (Bare et al., 1994; Zimprich et al., 1995). MOR-1B, initially isolated from the rat, differed from MOR-1 with the replacement of exon 4 by exon 5. At the protein level, the predicted 12 amino acids at the C terminus of MOR-1 were replaced by a different sequence of five amino acids, which functionally made rat MOR-1B (rMOR-1B) less sensitive to agonist-induced desensitization. MOR-1 splicing has now been observed in a variety of species. A number of additional variants involving splicing at the C terminus have been identified in mice and rats (Pan et al., 1999, 2000; Narita et al., 2003; Kvam et al., 2004; Pasternak et al., 2004), and humans (Pan et al., 2003, 2005). Similar to rMOR-1B, these variants have alternative exons in place of exon 4; however, splicing in the mouse is even more complex. A series of additional variants derived from splicing at the 5′-end of the mouse *Oprm* gene also have been described previously (Pan et al., 2001). We now report the identification of the mouse homolog of rMOR-1B and a series of additional variants derived from alternative splicing within the mouse exon 5.

Materials and Methods

Isolation of Genomic BAC Subclones Containing Exon 5. To identify the mouse homolog of the rat exon 5 in mouse *Oprm* gene, the mouse BAC clone A (Pan et al., 1999, 2000, 2001) containing exons 1 to 4 was digested with HindIII. The HindIII-digested fragments were then subcloned into the HindIII site of Bluescript SK vector. After being transformed into JM109-competent cells, the transformants were plated on Luria broth plates containing 0.2 mM isopropyl 1-thio-β-D-galactopyranoside, 0.008% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, and 100 μg/ml ampicillin for colony lifting. A 280-bp cDNA fragment of the rat exon 5 was obtained by reverse transcription (RT)-polymerase chain reaction (PCR) with rat brain total RNA, and a pair of primers from rat MOR-1B (sense primer, 5′-CCA CCA GAA AAT AGT ATT ATT TTG AAA AGG C-3′, and antisense primer, 5′-GTT CAT TGA GAG AAG CTT GCC CAG AGT CTG-3′) (Zimprich et al., 1995) was ^{32}P-labeled and used as a probe for colony hybridization. The colony-lifting and hybridization procedures were performed using the protocols described previously (Pan et al., 1999, 2000, 2001). Six positive clones with identical ~1-kb insertions were obtained. Sequence analysis of the fragment indicated that the clone contained the exon sequence, exon 5a, homologous to rat exon 5. The mouse exon 5a and rat exon 5 sequences shared 91% identity.

RT-PCR. Total RNA was extracted from mouse brain by the guanidinium thiocyanate-phenol-chloroform method (Pan et al., 1999, 2000, 2001) and reverse-transcribed with random hexamers and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). The first-strand cDNA was then used as template in PCR with a sense primer from exon 3 (5′-CCC AAC TTC CTC CAC AAT CGA A-3′) and antisense primer from exon 5a (5′-GGT GTG CTT CTC CCA GTT CTG TGT ATA-3′). Two fragments were amplified. Sequence analysis of the fragments revealed that one fragment contained exons 3 and 5a sequences and that the other had a new 699-bp sequence inserted between exons 3 and 5a. The new exon sequence (exon 5b) was mapped upstream of exon 5a by PCR and by sequencing the BAC clone A.

To obtain full-length cDNA clones of the potential exon 5-associated variants, two sense primers from the 5′-untranslated region of exon 1 (sense primer A, 5′-AGA GGA AGA GGC TGG GGC G-3′, and sense primer B, 5′-GGA ACC CGA ACA CTC TTG AGT GCT-3′), two antisense primers from exon 5a (antisense A, 5′-CTT GCC CCA GAG ACG GAA TGA TGC AG-3′, and antisense primer B, 5′-GGG GTT GGC ACC AGC ATT AGG TAC TC-3′), and two antisense primers from exon 5b sequence (antisense primer C, 5′-GGT CTT CCT TTG GAG TTC CAC AGG AAG-3′, and antisense primer D, 5′-TTG TAA TCT AAT CAC CAT GGA TTA GGT GCC-3′) were used in nested PCRs with the first-strand cDNA from mouse brain total RNA as template. Five fragments ranging from ~1.3 to ~3.0 kb long were obtained, subcloned into pCRII-TOPO vector (Invitrogen), and sequenced. Sequence analysis of the fragments indicated that all of the fragments contained exons 1, 2, and 3 but different downstream exon sequences that were all associated with exon 5a. A comparison of these downstream exon sequences with the genomic sequences from the BAC clone A (see below) indicated that all of the exon sequences were derived from a continuous genomic sequence, which was started from exon 5a and extended ~3.2 kb to its upstream. We refer to the entire sequence as exon 5 and the subsets of exon 5 as exons 5a, 5b, 5c, 5d, and 5e because of alternative splicing within exon 5 (Figs. 1 and 2). Based on the splicing pattern within exon 5, we named the five cloned full-length cDNAs mMOR-1B1, mMOR-1B2, mMOR-1B3, mMOR-1B4, and mMOR-1B5 (Figs. 1 and 2). We have sequenced the clones in both orientations with the appropriate primers. The sequences of exons 1 to 3 were identical to those of mMOR-1, and the exon 5 sequences were 100% aligned to those of the mMOR-1 gene in the Mouse Genome Database (Ensembl and NCBI).

Identification and Characterization of Genomic BAC Subclones Containing the New Exon Sequence. The BamHI- or KpnI- or SacI-digested BAC clone A fragments were subcloned into Bluescript SK vector. The resulting plasmids were screened through colony lifting and hybridization as mentioned above with two probes, one from the 108-bp sequence of exon 5c and the other from the 362-bp sequence of exon 5e. Four overlapping positive clones ranging from ~7 to ~10 kb long were obtained, and a 3.4-kb region of the overlapping clones containing all of the new exon 5s sequences was sequenced. The sequence was identical to that from public mouse genomic databases with the exception of one base mismatch in exon 5c and one more four-base GTTT repeat in exon 5b (Fig. 2), which may represent the difference between the strain mice.

Northern Blot Analysis. Northern blot analysis was performed as described previously (Pan et al., 1999, 2000, 2001). In brief, 20 μg of total RNA extracted from mouse brain was separated on a 0.8% formaldehyde agarose gel and transferred to GenePlus membrane (PerkinElmer Life and Analytical Sciences, Boston, MA). The membranes were hybridized with a 125-bp ^{32}P-labeled exon 5a probe produced by PCR (sense primer, 5′-CAG AAA ATA GAT TTA TTT TGA AAA GGC A-3′; antisense primer, 5′-GGG GTT GGC ACC AGC ATT AGG TAC TC-3′), a 273-bp ^{32}P-labeled exon 5b probe (sense primer, 5′-GCC TTG ATA ATT AGG GCA CCA AAG GGG-3′; antisense primer, 5′-CGG CTC AAT TCA CAG CTT TGG GCC-3′), a 300-bp ^{32}P-labeled exon 5c probe (sense primer, 5′-CAA GCC TCA CAC TTC AGT AAT GGA ATG-3′; antisense primer, 5′-CTT CTT CCA CCA AAG CCA GAC AGG C-3′), a 243-bp ^{32}P-labeled exon 5d probe (sense primer, 5′-GCA CAC CAA AAA CCT CAA GAA TGC CTG-3′; antisense primer, 5′-CCC ATT TCA GCA CTA TGA GAA GTT ATC-3′), or a 93-bp ^{32}P-labeled exon 5e probe (sense primer, 5′-GTG TAT GAG TGC TAT GCC CAC AGG G-3′; antisense primer, 5′- TTG CGG GGG GTG GGG GTG-3′).

Expression of the Variants. The cDNA fragments containing the full-length mMOR-1B1, mMOR-1B2, mMOR-1B3, mMOR-1B4, and mMOR-1B5 in pCRII-TOPO were subcloned into pcDNA3.1 or pcDNA5/FRT (Invitrogen) with the appropriate restriction enzymes. The resulting plasmids, mMOR-1B1/pcDNA3, mMOR-1B2/pcDNA3, mMOR-1B3/pcDNA3, mMOR-1B4/pcDNA5/FRT, and mMOR-1B5/pcDNA3, respectively, were transfected into Chinese hamster ovary (CHO) cells or CHO/Flip-In cells by Lipofectamine reagent (Invitrogen). Stable transformants were obtained 2 weeks after selection

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with G418 or hygromycin and screened in [^{3}H]DAMGO or [^{3}H]diprenorphine-binding assays (PerkinElmer Life and Analytical Sciences).

Relative Semiquantitative RT-PCR. Total RNA obtained from mouse brain as described above was treated with TURBO DNA-free reagents (Ambion, Austin, TX) and reverse-transcribed with random hexamers and Superscript II reverse transcriptase. Aliquots of the first-strand cDNA were used as templates in PCRs with sets of primers specific for each variant. The following PCR primers were used: mMOR-1B1, exon 3 sense primer A (5′-GCT GCC TGA ACC CAG TTC TTT ATG CG-3′) and an exon 5a antisense primer (5′-GGG GTT GGC ACC AGC ATT AGG TAC TC-3′); mMOR-1B2, exon 3 sense primer B (5′-GCA TCC CAA CTT CCT CCA CAA TCG AAC-3′) and an exon 5b antisense primer (5′-CCC CTT TGG TGC CCT AAT TAT CAA GGC-3′); mMOR-1B3, exon 3 sense primer A and an exon 5c antisense primer (5′-CTA GGT CTA GCT CAT GAA TGC TCT TTG GTT GG-3′); mMOR-1B4, exon 3 sense primer A and an exon 5d antisense primer (5′-CCC ATT TCA GCA CTA TGA GAA GTT ATC-3′); mMOR-1B5, exon 3 sense primer A and an exon 5e antisense primer (5′-TTG CGG GGG GTG GGG GTG GG-3′); and mMOR-1, exon 3 sense primer A and an exon 4 antisense primer (5′-GCA ACC TGA TTC CAA GTA GAT GGC AG-3′). PCRs were performed in a thermal cycler (PTC-200; MJ Research (Watertown, MA) using Platinum *Taq*DNA polymerase (Invitrogen) with an initial 2-min denaturing step at 94°C followed by 48 cycles, each containing a 20-s denaturing step at 94°C, a 20-s annealing step at 65°C, a 45-s of extension step at 72°C, and a final 3-min extension at 72°C. PCR products were separated on a 1.5% agarose gel. The gel then was stained with 0.02% ethidium bromide, and images were taken with a FluorChem 8000 (Alpha Innotech, San Leandro, CA). Relative band intensities were quantified with the AlphaEase FC software (Alpha Innotech). All of the PCR products showed bands with correct sizes predicted from the following primers: 295 bp for mMOR-1B1, 187 bp for mMOR-1B2, 362 bp for mMOR-1B3, 417 bp for mMOR-1B4, 266 bp for mMOR-1B5, and 278 bp for mMOR-1. Each band was purified, and the gel and its identity were confirmed by DNA sequencing. PCR controls without the template showed no visible bands (data not shown).

Receptor-Binding Assays. Membranes were prepared from cells stably transfected with the pcDNA3 constructs as described above. [^{3}H]DAMGO or [^{3}H]diprenorphine saturation and competition-binding assays were performed at 25°C for 60 min in 50 mM potassium phosphate buffer, pH 7.4, containing 5 mM magnesium sulfate (Pan et al., 1999, 2000, 2001). Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of 10 μM levallorphan. Protein concentrations were determined as described previously using bovine serum albumin as the standard (Pan et al., 1999, 2000, 2001).

[^{35}S]GTPγS-Binding Assay. [^{35}S]GTPγS binding was performed on membranes prepared from transfected cells in the presence and absence of the indicated opioid for 60 min at 30°C in the assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, 0.2 mM EGTA, and 10 mM NaCl) containing 0.05 nM [^{35}S]GTPγS and 30 μM GDP, as previously reported (Pasternak et al., 2004; Pan et al., 2005). After the incubation, the reaction was filtered through glass-fiber filters (Whatman Schleicher & Schuell, Keene, NH) and washed three times with 3 ml of ice-cold 50

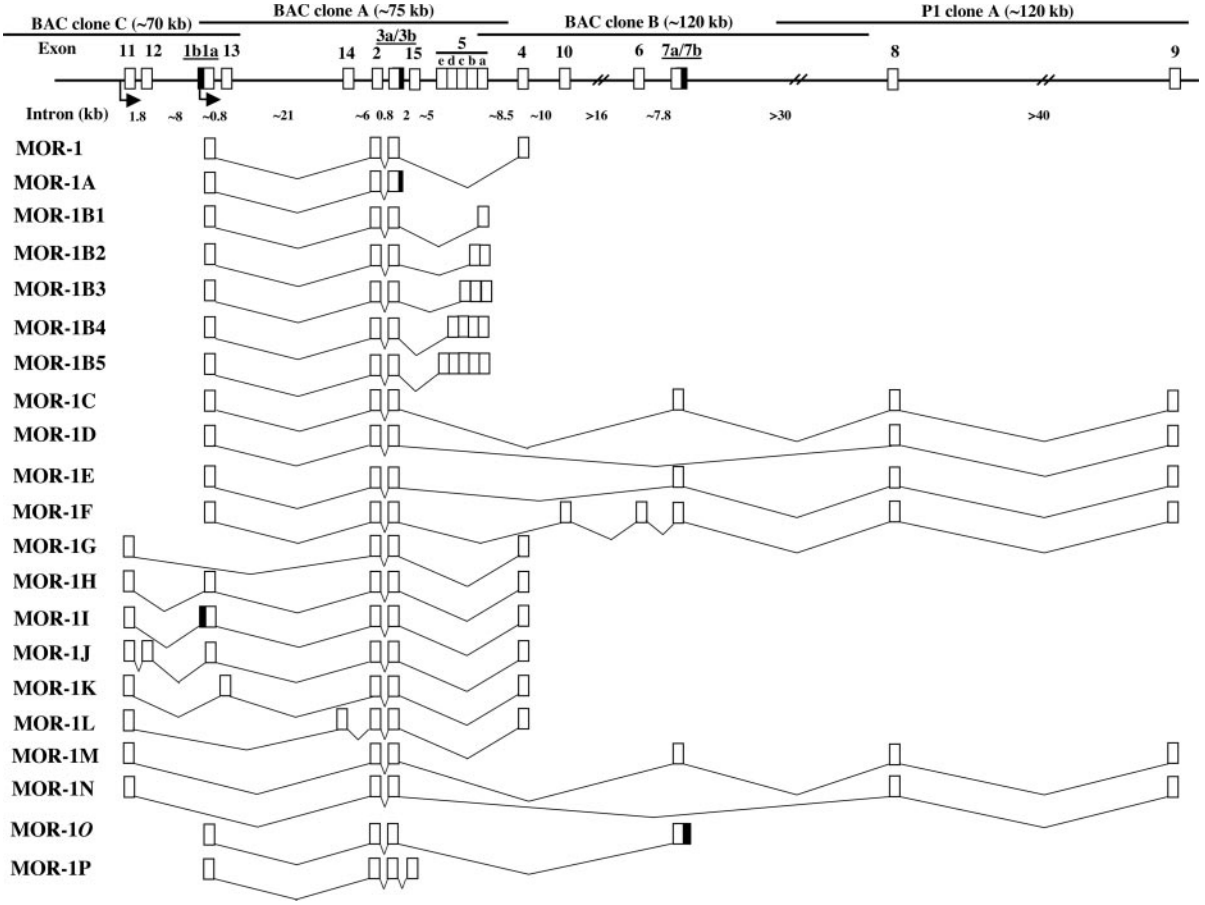

Fig. 1. Schematic of the mouse *Oprm* gene structure and alternative splicing. The schematic of the gene structure. Exons and introns are shown by boxes and horizontal lines, respectively. The transcriptional start points are indicated by arrows. The genomic BAC clones are shown by heavy horizontal lines on the top. The complete cDNA and deduced amino acid sequences of mMOR-1B1, mMOR-1B2, mMOR-1B3, mMOR-1B4, and mMOR-1B5 have been deposited in the GenBank database (accession numbers AF167566, AF167567, AF346813, AF346814, and AF346812, respectively).

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mM Tris-HCl, pH 7.4, on a semiautomatic cell harvester. Filters were transferred into vials with 5 ml of Liquiscent (National Diagnostics, Atlanta, GA), and the radioactivity in vials was determined by scintillation spectroscopy in a Tri-Carb 2900TR counter (PerkinElmer Life and Analytical Sciences). Basal binding was determined in the presence of GDP and the absence of drug.

Results

Cloning Exon 5 Splice Variants of the Mouse *Oprm* Gene. In the rat, rMOR-1B contains exon 5 in place of exon 4 (Fig. 1) (Zimprich et al., 1995). We obtained the mouse exon 5a sequence from the mouse genomic BAC clone A using a

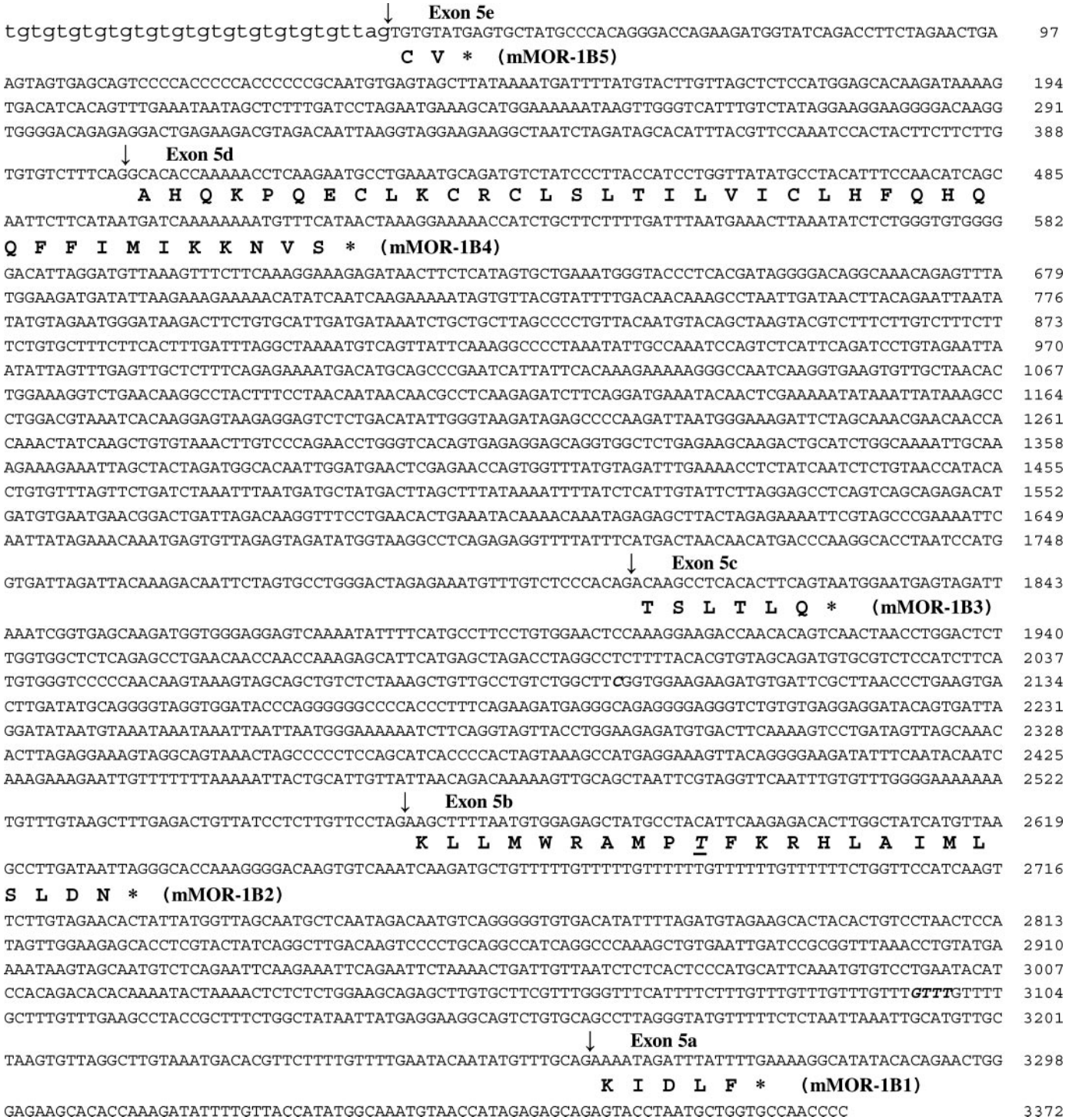

Fig. 2. Genomic sequence and predicted amino acid sequences of the exon 5 splice variants. The exon sequences are shown in capital letters, and partial intron sequences are indicated in lowercase letters. The predicted amino acids are shown in boldface capital letters. Termination codons are indicated by asterisk. The threonine, a potential protein kinase C phosphorylation site, from exon 5b is shown as italic and underlined. The one mismatch in exon 5c and one more GTTT repeat in exon 5b are shown as italic and boldface. The sequence has been deposited in GenBank under accession number AY390763.

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colony hybridization approach with a rat exon 5 probe. The mouse exon 5a sequence shared high homology (91% identity) with the rat exon 5. Subsequent RT-PCR with the primers from the mouse exons 3 and 5a revealed the anticipated splicing between exons 3 and 5a but also demonstrated a novel pattern of splicing upstream of exon 5a (Fig. 2). To generate full-length cDNAs, nested RT-PCR with primers from exons 1, 5a, and 5b led to the isolation of the full-length mMOR-1B2, mMOR-1B3, mMOR-1B4, and mMOR-1B5 clones as well as mMOR-1B1, which was homologous to the rat MOR-1B. Thus, exon 5 in the mouse contains five different 3′-splice sites linked to exon 3.

All of the mMOR-1B variants contained exons 1 to 3, differing only in their downstream sequence as a result of alternative splicing within exon 5 (Figs. 1 and 2). All of the splice junctions were consistent with the consensus splicing pattern. mMOR-1B1 had exon 5a as the fourth exon, which predicted the identical five amino acid sequence as reported in the rat rMOR-1B (Zimprich et al., 1995). mMOR-1B2 contained a 699-bp insertion (exon 5b) between exons 3 and 5a, which encoded 23 amino acids. In mMOR-1B3, splicing from exon 3 to exon 5c (108 bp) predicted six amino acids. Only two amino acids were predicted from exon 5e (362 bp) of mMOR-1B5 because of early termination of translation. On the other hand, exon 5d (362 bp) in mMOR-1B4 predicted 39 amino acids. Each of the amino acid sequences predicted from exon 5b to exon 5e was unique and differed from all of the previously identified C-terminal variants. The predicted amino acid sequence from exon 5b of mMOR-1B2 contained a protein kinase C phosphorylation site.

Northern Blot Analysis of the Variant mRNA. We next performed Northern blot analysis with various exon probes to estimate the relative size of the variant mRNAs (Fig. 3). All of the individual exon probes hybridized to a band of ~14 kb but with different intensities. The individual exon probes also revealed additional bands. For example, the exon 5b probe hybridized to a sharp strong band at approximately 11 kb and to a second diffuse band at 1.5 to 2.5 kb. A diffuse band in the 4- to 7-kb range was observed with all of the probes, although it was far more prominent with the exon 5c, 5d, and 5e probes. The different banding patterns illustrate the uniqueness of the various variant mRNAs and suggest that the individual exon probes associate with more than one transcript, as predicted from the structure.

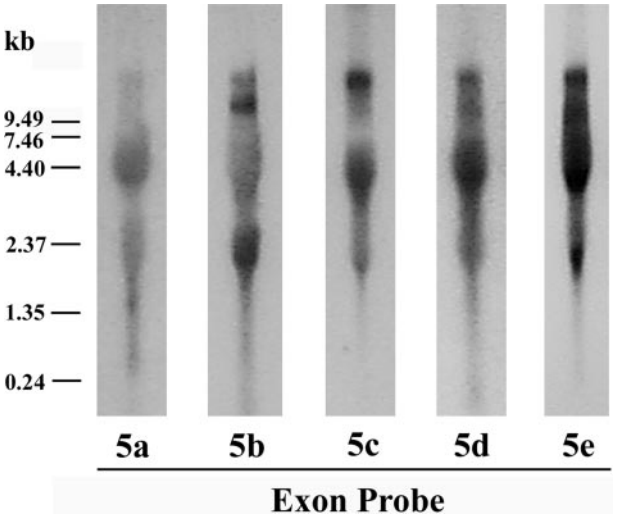

Fig. 3. Northern blot of exon 5 in mouse brain. Northern blot analysis was performed on mRNA isolated from whole brain as described previously (Pan et al., 1999, 2000, 2001). In brief, 20 μg of total RNA extracted from mouse brain was separated on a 0.8% formaldehyde agarose gel and transferred to GenePlus membrane. The membranes were hybridized with a 125-bp ^{32}P-labeled exon 5a probe, a 273-bp ^{32}P-labeled exon 5b probe, a 300-bp ^{32}P-labeled exon 5c probe, a 243-bp ^{32}P-labeled exon 5d probe, or a 93-bp ^{32}P-labeled exon 5e probe, as described under *Materials and Methods*.

Relative Abundance of the Variants. To estimate relative abundance of each variant mRNA, we performed semiquantitative RT-PCR using mouse brain total RNA. The results showed that mRNAs of all of the mMOR-1B variants were significantly lower than that of mMOR-1 ($p < 0.001$), ranging from 18 to 39% mMOR-1 level (Fig. 4, a and b). However, it should be pointed out that the band amplified by the exons 3 and 4 primers to estimate mMOR-1 levels might also have amplified six other splice variants (mMOR-1G to mMOR-1L) generated from the exon 11 promoter that are

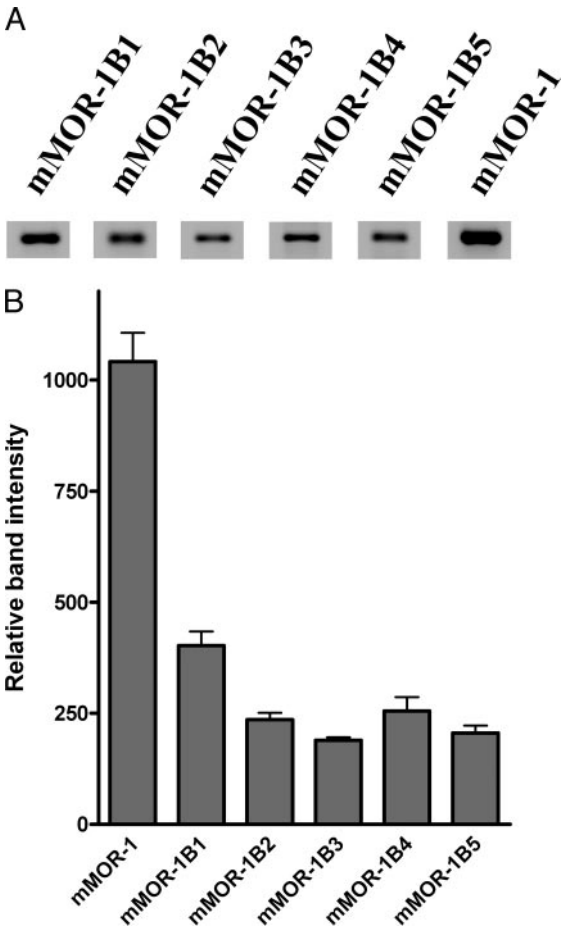

Fig. 4. Semiquantitative RT-PCR of the mMOR-1B variants. A, RT-PCR was performed as described under *Materials and Methods*. Agarose gel stained with ethidium bromide was photographed with FluorChem 8000 system, as described under *Materials and Methods*. B, relative band intensities from the gel were quantified with AlphaEase FC software. Bars represent the mean ± S.E. of the relative band intensity from three independent experiments. Relative intensity values were as follows: mMOR-1, 1042 ± 65; mMOR-1B1, 403 ± 31; mMOR-1B2, 236 ± 15; mMOR-1B3, 189 ± 7; mMOR-1B4, 255 ± 31; and mMOR-1B5, 205 ± 17. Significance was assessed using ANOVA. Overall, the values were significant different ($p < 0.001$). Tukey post hoc analysis revealed that mMOR-1 differed from all of the individual variants ($p < 0.001$). mMOR-1B1 also differed from mMOR-1B3 ($p < 0.01$) and mMOR-1B5 ($p < 0.05$), and mMOR-1B2 also differed from mMOR-1 ($p < 0.001$).

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also present but at lower expression levels than mMOR-1 itself (Pan et al., 2001). Thus, all of these relative values must be considered as only estimates of the expression of the variants.

Receptor Binding of the Variants. Earlier studies from our laboratory revealed that the mouse, rat, and human C-terminal splice variants of MOR-1 retained their μ-selectivity and affinity (Pan et al., 1999, 2000, 2001, 2005; Pasternak and Pan, 2004; Pasternak et al., 2004). To assess the new variants, we cloned their full-length cDNAs downstream of a cytomegalovirus promoter in the mammalian expression vector pcDNA3 and established stably transfected CHO cell lines. In saturation studies, the agonist [^{3}H]DAMGO labeled all of the variants with similar high affinities (Table 1) with the exception of mMOR-1B4. The expression levels of the different variants were also comparable.

Competition studies using the agonist [^{3}H]DAMGO confirmed the μ-selectivity of all of the variants (Table 2). The μ-ligands competed binding very potently, whereas the κ_1-selective opioid U50,488H and the δ-selective ligand DPDPE were ineffective. Several of the drugs showed differences in their K_i values among the variants, but these differences were modest. DADLE showed the greatest differences, which were predominantly attributed to mMOR-1B1. Within a variant, we also saw a modest range of affinities for the different drugs.

Binding determined with radiolabeled opioid agonists and antagonists differ (Pert et al., 1973; Pasternak and Snyder, 1975b; Pasternak et al., 1975; Snyder and Pasternak, 2003), presumably because agonists label only the agonist receptor conformation, whereas antagonists bind to both agonist and antagonist conformations. This is clearly shown when observing the B_{max} values for mMOR-1 using the agonist [^{3}H]DAMGO and the antagonist [^{3}H]diprenorphine. Here, the B_{max} value for [^{3}H]diprenorphine bound with high affinity (K_D 0.71 ± 0.18 nM; n = 3) with a B_{max} (1.64 ± 0.13 pmol/mg of protein; n = 3) that was approximately 10-fold greater than the B_{max} seen with the agonist [^{3}H]DAMGO in membranes from the same cells (0.16 ± 0.04 pmol/mg of protein) (Table 1). This implies that the vast majority of the sites expressed in the cell line are in an antagonist conformation. Although antagonist conformations predominate, even in brain (Pasternak and Snyder, 1975a), this may be even more pronounced in cells overexpressing the receptors. The differences also are evident when examining the K_i values of opioids for mMOR-1 (Table 3). K_i values with [^{3}H]DAMGO provide an indication of the affinity of the competitor for the agonist state, whereas the values against [^{3}H]diprenorphine provide an estimate of the affinity for the antagonist state because the vast majority of sites labeled by the radiolabeled antagonist are in the antagonist conformation. The K_i value for morphine is shifted by almost 20-fold, whereas M6G is shifted by more than 100-fold. The antagonist naloxone shows little difference between the two radioligands.

The binding profile of mMOR-1B4 was quite unique (Table 3). Attempts to screen stable CHO or human embryonic kidney 293 clones transfected with mMOR-1B4 failed to identify any with specific [^{3}H]DAMGO binding. We then examined the binding with the antagonist [^{3}H]diprenorphine, which labeled mMOR-1B4–transfected CHO cells with an affinity (K_D) of 1.4 ± 0.36 nM (n = 3), which was comparable with its affinity for mMOR-1 sites. The expression of mMOR-1B4 in the cells, measured with [^{3}H]diprenorphine, was lower than that of mMOR-1, although it remained relatively robust, with a B_{max} value of 0.68 ± 0.03 pmol/mg of protein (n = 3). This level of binding is greater than that seen with the agonist [^{3}H]DAMGO in the other transfected cell lines (Table 1).

The overall binding profile of mMOR-1B4 differed from the other 10 full-length mMOR-1 variants. This was shown by

TABLE 1

[^{3}H]DAMGO saturation studies in mMOR-1 variants

[^{3}H]DAMGO saturation studies were performed in membranes of CHO cells stably expressing the indicated variant as described under *Materials and Methods*. K_D values were determined by nonlinear regression analysis using Prism (GraphPad Software Inc., San Diego, CA). Results are the mean ± S.E. of at least three independent determinations.

Clone	K_D	B_{max}
	nM	*pmol/mg protein*
mMOR-1	1.75 ± 0.44	0.16 ± 0.04
mMOR-1B1	1.20 ± 0.33	0.08 ± 0.01
mMOR-1B2	1.99 ± 0.29	0.12 ± 0.01
mMOR-1B3	0.77 ± 0.11	0.11 ± 0.02
mMOR-1B5	1.60 ± 0.41	0.11 ± 0.02

TABLE 2

Competition of [^{3}H]DAMGO binding in cells stabling expressing mMOR-1 variants

[^{3}H]DAMGO binding was performed in membranes from CHO cells stably transfected with the indicated variant constructs. K_i values were determined from at least three independent determinations of IC_{50} values (Cheng and Prusoff, 1973). Comparisons of the K_i values for each drug were then compared among the variants using ANOVA. Of the compounds, only DADLE ($P < 0.0001$), DSLET ($P < 0.04$), and endomorphin 2 ($P < 0.01$) showed significant differences. Tukey post hoc analysis for DADLE revealed that the K_i value for mMOR-1B1 differed significantly from mMOR-1, mMOR-1B3, and mMOR-1B5 ($P < 0.001$) as well as mMOR-1B2 ($P < 0.05$). With DSLET, mMOR-1B2 differed significantly from both mMOR-1B1 and mMOR-1B3 ($P < 0.05$), whereas mMOR-1B1 differed from both mMOR-1 and mMOR-1B3.

Ligand	K_i Value					ANOVA
	mMOR-1	mMOR-1B1	mMOR-1B2	mMOR-1B3	mMOR-1B5	
			nM			
Morphine	5.3 ± 2.0	5.3 ± 1.0	3.9 ± 0.4	1.5 ± 0.5	1.4 ± 0.6	N.S.
M6G	5.2 ± 1.8	10.1 ± 1.6	8.4 ± 1.3	3.9 ± 1.3	5.2 ± 0.1	N.S.
DAMGO	1.8 ± 0.5	1.4 ± 0.2	1.3 ± 0.1	1.8 ± 0.9	1.0 ± 0.3	N.S.
DADLE	2.1 ± 0.3	9.0 ± 1.1	4.9 ± 0.7	1.9 ± 0.4	2.4 ± 0.4	$P < 0.001$
DSLET	12.5 ± 3.6	8.9 ± 1.1	22.1 ± 1.5	8.7 ± 1.8	11.5 ± 5.2	$P < 0.04$
Naloxone	4.3 ± 0.9	0.5 ± 0.3	1.3 ± 0.1	1.4 ± 0.5	0.6 ± 0.3	N.S.
Dynorphin A	10.9 ± 0.5	14.6 ± 7.1	34.3 ± 18.4	8.7 ± 1.8	8.9 ± 2.3	N.S.
β-Endorphin	10.8 ± 2.9	6.8 ± 3.2	4.9 ± 1.7	3.1 ± 1.4	5.7 ± 1.2	N.S.
Endomorphin 1	2.1 ± 0.8	10.8 ± 5.6	5.0 ± 1.8	3.2 ± 0.6	4.3 ± 0.8	N.S.
Endomorphin 2	4.2 ± 1.8	12.4 ± 1.5	8.4 ± 1.1	3.2 ± 0.8	10.6 ± 1.8	$P < 0.01$
U50,488H	>500	>500	>500	>500	>500	
DPDPE	>500	>500	>500	>500	>500	

N.S., not significant; DSLET, [D-Ser^2,Leu^5]enkephalin-Thr^6.

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the [^{3}H]diprenorphine competition studies in mMOR-1- and MOR-1B4–expressing cells (Table 3). The binding to the MOR-1B4 cells was μ-selective, as shown by the poor affinity of the κ- and δ-antagonists nor-BNI and naltrindole. However, the affinity of many agonists in the mMOR-1B4 cells was far lower than in mMOR-1-expressing cells, with K_i values for most over 100 nM. The poor affinity of DAMGO in the competition studies explains why attempts to demonstrate [^{3}H]DAMGO binding directly were not successful. Several agonists stood out from the others. Etorphine, etonitazene, [DMT^1]DALDA, and buprenorphine all retained high affinity in the mMOR-1B4 cells, with K_i values under 15 nM. Indeed, these ultrapotent analgesics bound to the mMOR-1B4 cell membranes with higher affinity than some of the antagonists. From a behavioral standpoint, these drugs are notable for their extremely high analgesic potency, yet not all of the highly potent μ-opioids retained high affinity, as illustrated by fentanyl, with a K_i value higher than 100 nM.

To assess whether the mMOR-1B4 sites labeled by [^{3}H]diprenorphine represented antagonist conformations of the receptor, we explored the effects of the stabilized GTP analog guanosine Gpp(NH)p on the binding in both mMOR-1 and mMOR-1B4 cells (Table 4). GTP and its analogs selectively diminish the affinity of agonists (Childers and Snyder, 1978). In the current study, we saw agonist shifts but they remained relatively modest. The antagonists naloxone and CTAP showed no shift in the mMOR-1 cells. However, they did reveal a small shift with mMOR-1B4. Although this shift is very modest, it raises the possibility that they may be partial agonists at this variant. Overall, the small shifts for the agonists is consistent with the earlier studies, implying that the vast majority of the sites in both the mMOR-1 and mMOR-1B4 cells are in an antagonist conformation.

Characterization of the Variants by [^{35}S]GTPγS-Binding Assay. Prior studies have documented functional differences among a number of MOR-1 variants (Bolan et al., 2004; Pasternak et al., 2004; Pan et al., 2005). Therefore, we examined the ability of the mMOR-1B variants to activate G-proteins using a [^{35}S]GTPγS-binding assay (Table 5). The range of the EC_{50} values differed from one variant to another. Some showed little difference among the drugs, whereas mMOR-1B1 revealed a greater range. For most of the drugs, their relative EC_{50} values in the [^{35}S]GTPγS stimulation assay did not correlate well with their relative receptor-binding affinities. Although the K_i and EC_{50} values for M6G ($r^2 = 0.72$) and for endomorphin 2 ($r^2 = 0.73$) showed a modest correlation, the correlations for the remainder of the drugs were very poor, as was the correlation for all of the drugs together ($r^2 = 0.16$).

The EC_{50}/K_i ratio provides an indication of the ability of the drug to activate the receptor relative to its receptor occupancy (Table 5). Because the conditions used in the receptor binding and [^{35}S]GTPγS-binding assays were not identical, this ratio should not be considered a direct indication of intrinsic activity, but it still is useful in evaluating the drugs. The ratios within a given variant ranged up to 10-fold, and the rank order of the ratios of the drugs to each other varied among the splice variants. For example, endomorphin 2 had the lowest ratio for mMOR-1B5 and the highest ratio for mMOR-1B3. M6G was among the lowest for mMOR-1B5 and the highest against mMOR-1B1.

For many of the variants, the maximal stimulation varied

TABLE 3

Receptor binding of [^{3}H]DAMGO and [^{3}H]diprenorphine binding in mMOR-1 and mMOR-1B4

[^{3}H]Diprenorphine binding was performed in membranes from CHO cells stably transfected with the indicated variant constructs. K_i values were determined from at least three independent determinations of IC_{50} values (Cheng and Prusoff, 1973). K_i values for [^{3}H]DAMGO are from TABLE 2.

Ligand	mMOR-1		mMOR-1B4
	[^{3}H]DAMGO	[^{3}H]Diprenorphine	[^{3}H]Diprenorphine
		nM	
Morphine	5.3 ± 2.0	94.9 ± 18.3	349 ± 79
M6G	5.2 ± 1.8	609.3 ± 110	>1000
DAMGO	1.8 ± 0.5	50.2 ± 2.5	208 ± 23
DADLE	2.1 ± 0.3	137 ± 13	600 ± 48
Endomorphin 1	2.1 ± 0.8	56.4 ± 7.4	>1000
Etorphine		0.7 ± 0.2	6.7 ± 0.4
Fentanyl		48.5 ± 8.0	137 ± 22
Etonitazene		3.0 ± 0.7	6.6 ± 0.5
β-Endorphin	10.8 ± 2.9	34.6 ± 5.5	873 ± 116
Dynorphin A	10.9 ± 0.5	127 ± 29	>1000
NalBzoH		1.9 ± 0.3	5.4 ± 2.0
[Dmt^1]DALDA		2.1 ± 0.4	13.6 ± 5.3
Nalorphine		27.4 ± 6.0	80 ± 31
Buprenorphine		0.4 ± 0.1	0.8 ± 0.2
Naloxone	4.3 ± 0.9	8.2 ± 1.0	17.6 ± 1.8
Diprenorphine		1.0 ± 0.3	2.4 ± 0.2
Naltrexone		1.4 ± 0.1	6.1 ± 0.4
CTAP		1.9 ± 0.2	8.0 ± 1.3
CTOP		3.5 ± 0.3	18.8 ± 4.3
Nor-BNI		188 ± 28	267 ± 34
Naltrindole		66.7 ± 3.3	198 ± 33
U50,488H	>500	>1000	>1000
DPDPE	>500	>1000	>1000

CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; NalBzoH, naloxone benzoylhydrazone.

TABLE 4

Effect of guanosine 5′-(β,γ-imido)triphosphate on [^{3}H]diprenorphine binding in mMOR-1 and mMOR-1B4

[^{3}H]Diprenorphine binding was performed in membranes from the stably transfected CHO cells with or without 100 μM GppNHp. K_i values were determined from at least three independent determinations. Student's t test was used to compare the K_i values between the groups with and without GppNHp for each drug.

	mMOR-1			mMOR-1B4		
	Control	With Gpp(NH)p	Shift	Control	With Gpp(NH)p	Shift
			nM			
Morphine	104.3 ± 2.2	147.8 ± 8.1*	1.4	296 ± 21	949 ± 29**	3.2
DAMGO	55.5 ± 1.3	82.8 ± 10.8	1.5	343 ± 161	908 ± 304	2.6
Etorphine	0.49 ± 0.06	0.55 ± 0.04	1.1	2.1 ± 0.4	4.6 ± 0.6	2.2
Fentanyl	53.6 ± 4.0	89.3 ± 7.4	1.7	446 ± 130	659 ± 56	1.5
Naloxone	15.4 ± 1.6	14.1 ± 0.7	0.9	20.9 ± 1.1	37.3 ± 10.9	1.8
CTAP	3.7 ± 0.5	2.1 ± 0.4**	0.6	8.9 ± 3.1	10.8 ± 5.8	1.2

Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate.
* $P < 0.05$.
** $P < 0.01$.

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TABLE 5
Stimulation of [^{35}S]GTPγS binding in mMOR-1 splice variants
Basal [^{35}S]GTPγS binding was assessed in membranes from cells stably transfected with the indicated variants after stimulation of [^{35}S]GTPγS binding by the indicated drug. The maximal stimulation, defined as the percentage increase over basal binding, and the dose of drug needed to elicit 50% of the maximal response (EC_{50}) were calculated as described under *Materials and Methods*. Results are the means ± S.E.M. of at least three independent determinations.

Ligand	mMOR-1			mMOR-1B1			mMOR-1B2			mMOR-1B3			mMOR-1B5		
	EC_{50}	EC_{50}/K_i	% Max	EC_{50}	EC_{50}/K_i	% Max	EC_{50}	EC_{50}/K_i	% Max	EC_{50}	EC_{50}/K_i	% Max	EC_{50}	EC_{50}/K_i	% Max
DAMGO	68 ± 4	38	100	39 ± 8	28	100	85 ± 18	65	100	100 ± 14	56	100	89 ± 13	89	100
Morphine	23 ± 2	4.3	102 ± 5	100 ± 38	18.9	104 ± 38	76 ± 13	19.5	82 ± 8	51 ± 6	34	91 ± 3	53 ± 4	37.9	87 ± 7
M6G	75 ± 18	11.7	122 ± 9	527 ± 245	52.2	117 ± 15	150 ± 6	17.9	71 ± 5	83 ± 19	21.2	82 ± 7	85 ± 18	16.3	88 ± 9
Dynorphin A	34 ± 9	3.2	109 ± 7	137 ± 69	9.4	83 ± 23	210 ± 25	6.1	81 ± 6	147 ± 56	16.9	90 ± 6	197 ± 32	22.4	75 ± 3
β-Endorphin	64 ± 7	7.6	97 ± 2	113 ± 47	16.6	69 ± 21	163 ± 22	33.3	84 ± 5	75 ± 19	24.1	93 ± 2	83 ± 27	14.6	80 ± 4
Endomorphin 1	26 ± 4	12.3	98 ± 8	57 ± 23	5.3	68 ± 19	126 ± 29	25.2	82 ± 8	99 ± 1	30.9	97 ± 2	89 ± 13	20.7	86 ± 7
Endomorphin 2	72 ± 11	17.1	124 ± 8	197 ± 95	15.9	90 ± 0	187 ± 23	22.3	92 ± 4	110 ± 6	34.4	80 ± 3	155 ± 8	14.6	82 ± 4

little among the drugs (Table 5). mMOR-1B1 had the greatest variation, with responses ranging from 117% DAMGO to only 68% endomorphin 1. DAMGO was the most efficacious among the all of the mMOR-1B variants, with the exception of mMOR-1B1. The most intriguing observation was that the relative efficacy of the drugs to each other varied from one variant to another. Among the mMOR-1B variants, M6G was the most efficacious with mMOR-1B1 and the least efficacious against mMOR-1B2. In contrast, endomorphin 1 was the lowest against mMOR-1B1 and among the highest with mMOR-1B3. Thus, the C-terminal differences of these variants were associated with varying effects on efficacy and potency of the μ-drugs.

As noted earlier, mMOR-1B4 proved to be a very unique variant. Although μ-opioid antagonists retained high affinity for this variant, the affinity of agonists in the binding was far lower (Table 3). Yet a number of the compounds were active in [^{35}S]GTPγS-binding assays, although their level of stimulation was lower than that seen with the other variants (Table 6). Of the active drugs, dynorphin A stimulated binding the most followed by etonitazene, fentanyl, and etorphine. As with the other variants, the maximal stimulation induced by the agonists did not correlate with their affinity for the receptors. Dynorphin A displayed very poor affinity in the binding assays, yet it was the most effective in the [^{35}S]GTPγS-binding study. Likewise, fentanyl displayed an affinity 20-fold lower than either etorphine or etonitazene but stimulated [^{35}S]GTPγS binding as well.

To determine whether or not the stimulation was opioid-specific, we examined the sensitivity of both etorphine and etonitazene to several opioid antagonists (Fig. 5). The maximal stimulation of both agents at 1 μM was modest but similar to values seen at 25 μM. Etorphine stimulated binding by only 27 ± 3% and etonitazene by only 35 ± 6%. The stimulated binding of both opioids was reversed by the opioid-selective antagonists, naloxone, naltrexone, and diprenorphine, confirming its specificity.

Discussion

The rMOR-1B variant was the first variant identified in the rat (Zimprich et al., 1995) and was recently reported in the mouse (Narita et al., 2003). Other splice variants of the mouse *Oprm* gene have also been reported, many of which are alternatively spliced downstream of exon 3 (Pan et al., 1999, 2000, 2001, 2005; Kvam et al., 2004; Pasternak and

TABLE 6
Stimulation of [^{35}S]GTPγS binding in stably mMOR-1B4-transfected CHO cells
Basal [^{35}S]GTPγS binding was assessed in membranes from cells stably transfected with the indicated variants followed by the stimulation of [^{35}S]GTPγS binding by the indicated drug at 25 μM. The stimulation was defined as the percentage increase over basal binding. Results are the means ± S.E.M. of at least three independent determinations.

Ligand	Stimulation of [^{35}S]GTPγS Binding
	%
Morphine	16.7 ± 2.8
DAMGO	25.9 ± 2.6
Etorphine	27.6 ± 7.0
Fentanyl	31.7 ± 12.2
Etonitazene	34.6 ± 5.8
β-Endorphin	17.5 ± 2.6
Dynorphin A	38.5 ± 10.4

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Pan, 2004). The present study describes five mouse variants generated from splicing within the mouse exon 5. The predicted amino acid sequences downstream from exon 3 for each of these variants differed in both composition and length, ranging from 2 to 39 amino acids. Together with the variants previously isolated, there are now 12 full-length and functional C-terminal variants of the mouse *Oprm* gene.

Similar to the other full-length MOR-1 variants, all of the full-length MOR-1B variants were μ-selective in binding assays, which was anticipated because the binding pocket was defined by exons 1, 2, and 3, which are identical in all of the variants. Therefore, functional differences must be due to the amino acid sequence differences at the tip of the C terminus. Similar to the other full-length variants (Bolan et al., 2004; Pasternak et al., 2004; Pan et al., 2005), the mMOR-1B splice variants differed in both the relative potency and efficacy of a number of μ-opioids in stimulating [^{35}S]GTPγS binding, a measure of G-protein activation. Differences in the relative efficacy of the drugs to each other and in their relative potency from variant to variant raise interesting questions. It is presumed that drugs administered in vivo will interact with all of the variants. However, the relative activation of one splice variant to another will probably differ from one drug to the next, leading to differences in their overall activation profiles. This may help explain the subtle but potentially significant behavioral differences among these agents.

Of the exon 5-containing variants, mMOR-1B4 clearly stands apart from the rest. Although its μ-selectivity was clearly demonstrated by the [^{3}H]diprenorphine competition studies, direct binding studies with [^{3}H]DAMGO were unsuccessful, presumably because of its low affinity. Most agonists displayed poor affinities against [^{3}H]diprenorphine binding in both mMOR-1- and mMOR-1B4-expressing cells, presumably because of the predominance of sites labeled by the radiolabeled antagonist in an antagonist conformation. The nearly 20-fold affinity shift of morphine, the 100-fold shift for M6G, and the 25-fold shift for DAMGO in mMOR-1 cells were anticipated. Yet the affinities of most of the agonists were even lower in the mMOR-1B4 cells. It is noteworthy that several agonists stood out from the others. Etorphine, etonitazene, [DMT^1]DALDA, and buprenorphine all retained high affinity for both variants. From a pharmacological standpoint, all of these drugs are very potent analgesics (Blane et al., 1967; Schiller et al., 2000; Neilan et al., 2001), raising the interesting possibility that their enhanced analgesic activity might result from a unique ability to interact with receptors in both agonist and antagonist conformations.

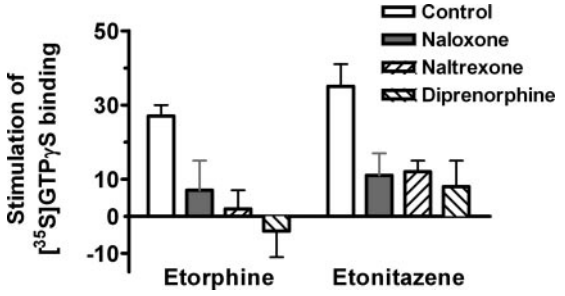

Fig. 5. Opioid-induced stimulation of [^{35}S]GTPγS binding in mMOR-1B4 and its sensitivity to antagonists. [^{35}S]GTPγS binding was determined in membranes from CHO cells stably transfected with mMOR-B4 that were treated with either 1 μM etorphine or 1 μM etonitazene alone or in the presence of 10 μM naloxone, 10 μM naltrexone, or 10 μM diprenorphine. The increase in stimulation is expressed as the percentage increase over basal levels. Results are the means ± S.E.M. of at least three independent determinations. ANOVA revealed differences between the agonist alone and the antagonists for both etorphine ($p < 0.033$) and for etonitazene ($p < 0.031$).

Why mMOR-1B4 displayed this unique binding profile remains unclear. It has 39 amino acids at the tip of the C terminus instead of the 12 amino acids encoded by exon 4. Yet the increased length of the tail alone cannot be responsible for its unusual characteristics because several other variants with even longer sequences, mMOR-1C with 52 amino acids and mMOR-1F with 58, have typical binding affinities for the μ-opioids (Pan et al., 1999, 2000). Thus, the distinct properties of mMOR-1B4 must reside in the sequence of the 39 amino acids. Because the binding pocket, which is defined by the transmembrane domains, is identical to that of the other full-length mMOR-1 variants, the binding and pharmacological differences may reflect the presence or absence of interactions of the C terminus with other membrane and/or cellular proteins. The presence of four cysteine residues within the 39 amino acid C terminus of mMOR-1B4 raises additional possibilities regarding its structure and interactions with other proteins.

A number of proteins associated with G-protein coupled receptors can modulate binding and function, including G-proteins, receptor (calcitonin) activity modifying proteins (Morfis et al., 2003), or regulators of G-protein signaling (De Vries et al., 2000; Sierra et al., 2002; Zachariou et al., 2003). Receptor dimerization also influences binding and function (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Jordan and Devi, 1999; George et al., 2000; Pan et al., 2002). Could the absence of a needed G-protein be responsible for the inability of mMOR-1B4 to show high affinity agonist binding? The repertoire of G-proteins varies among cell lines. CHO cells used in the current study reportedly lack $G_{i\alpha1}$, but transfection of $G_{i\alpha1}$ into the cells expressing mMOR-1B4 did not change any of the binding parameters (data not shown). We also expressed mMOR-1B4 in human embryonic kidney 293 cells to determine whether different cellular environments might restore agonist binding but without success (data not shown). Overexpression alone seems unlikely to explain our findings, because the expression levels of mMOR-1 were even greater than mMOR-1B4. Other receptors also can modulate function. For example, some receptors lack function unless coexpressed with a second one, as seen with the GABA type B ($GABA_B$) receptors (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998). In other situations, heterodimerization may change the pharmacology of the complexed receptors compared with the individual receptors expressed alone, as shown for several pairs of opioid receptors (Jordan and Devi, 1999; George et al., 2000; Pan et al., 2002). All of these possibilities must be considered.

MOR-1 splice variants also display regional distributions distinct from those of MOR-1 at the regional, cellular, and ultracellular levels (Abbadie et al., 2000a,b, 2001). Likewise, the regional distribution of mMOR-1B4 was unlike any others, including rMOR-1B (Y. Zhang, X. Y. Pan, and G. W. Pasternak, manuscript in preparation). Within the spinal cord, it weakly labeled the dorsal horn, robustly labeled Onuf's nucleus at the L5 and L6 levels, and diffusely labeled the anterior horn. Within the brain, the most intense labeling was observed within the Purkinje cells of the cerebellum, with some labeling in the olfactory bulb. In the rat, rMOR-1B

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has an unusual immunohistochemical labeling pattern within the brain, with high levels of labeling restricted to the olfactory bulb and little labeling in areas typically associated with opioid modulation of pain (Schulz et al., 1998).

These MOR-1 splice variants may help explain many of the clinical and behavioral observations seen μ-opioids. Both clinical and preclinical studies reveal subtle differences among the μ-opioids. Except for mMOR-1B4, μ-opioids label all of the full-length variants with similar affinities. However, the overall pharmacological response of a drug reflects the summation of the activation of all of the MOR-1 variants bound. Because both the efficacy and potency of each drug varies from variant to variant and does not correlate with binding affinity, the overall pharmacological activation profiles of each μ-drug would be expected to differ. This ability to dissociate efficacy and potency from binding also opens the possibility of novel drugs. Selectivity among the MOR-1 variants is not likely to be achieved based on the binding affinities alone, because their binding pockets are all structurally identical. However, it may be possible to obtain selectivity of action by independently modulating the efficacy and/or potency of the compounds at the different variants.

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