Human Opioid Receptors: Chromosomal Mapping and mRNA Localization

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INTRODUCTION

The opioid system modulates complex processes such as neuroendocrine function, analgesia, immunity, and cardiovascular regulation. A challenge in the field of neuroscience has been the molecular characterization of the opioid receptors. The recent isolation and sequencing of the genes and cDNAs encoding the opioid receptors permit studies of structure, function, and the cellular localization of these receptors. This chapter describes recent studies on the characterization and chromosomal mapping of the human opioid receptor genes and the localization of human opioid receptor messenger ribonucleic acid (mRNA) in the human brain.

MULTIPLE OPIOID RECEPTORS

Pharmacological studies using opioid peptides and alkaloid ligands have identified several classes of opioid receptor including the mu (μ), delta (d), and kappa (k) (Lord et al. 1977), (Martin et al. 1976). The first opioid receptor cloned was the mouse d receptor reported simultaneously by Evans and colleagues (1992) and Kieffer and colleagues (1992). This receptor was isolated by ligand screening of eukaryotic expression libraries. Subsequently the rat μ and d receptors were isolated by low stringency hybridization using probes derived from the d receptor cDNA (Chen et al. 1993; Fukuda et al. 1993). The mouse k receptor cDNA was isolated by polymerase chain reaction (PCR) using degenerate oligo-nucleotide primers to the structurally similar somatostatin receptor (Yasuda et al. 1993). Multiple similar cDNA clones for the μ, d, and k opioid receptors from several species have since been identified. Although subtypes for all three classes of opioid receptors have been proposed based on pharmacological studies, thus far only a single member from each class of opioid receptor has been cloned (Mattia et al. 1992; Pasternak 1986; Xu et al. 1991; Zukin et al. 1988). The fact that only one gene

locus was identified for each class of opioid receptors suggests that the subtypes identified by pharmacological studies may result from posttranslational, splicing modifications or differential interactions with associated proteins.

The cloning and sequencing of the opioid receptors also verified that these receptors belonged to the family of guanosine triphosphate binding protein (G-protein) receptors. G-protein receptors share some structural features such as seven alpha-helical hydrophobic domains that form the membrane spanning regions and methods of signal transduction that involve activation of specific G-proteins (Lameh et al. 1990; Strosberg 1991). It is the membrane-spanning regions and cytoplasmic loops that share the greatest sequence similarities between the classes of opioid receptors. The conservation of these regions within the family of opioid receptor genes may suggest overlapping functional and structural units of the opioid receptors (Douglass et al. 1984; Hershey et al. 1991; Traut 1988).

Several investigators have recently identified an additional G-protein receptor in rat (Bunzow et al. 1994; Chen et al. 1994; Fukuda et al. 1994; Wang et al. 1994a) and human brain (Keith et al. 1994; Mollereau et al. 1994) that has a high homology to the opioid receptor gene family. Although this receptor is not a μ, d, or k subtype, it shares significant homology to these opioid receptors at the amino acid level, particularly in the regions of the cytoplasmic loops and transmembrane domains. The abundant distribution of this receptor in structures of the hypothalamic-pitutary axis and cortical, spinal, and limbic nuclei suggests a role in neuroendrocrine function and the mediation of pain perception and analgesia (Bunzow et al. 1994; Keith et al. 1994; Wang et al. 1994a). Unfortunately, the lack of identification of the endogenous ligand for this receptor has delayed further characterization of the role of this relative of the opioid receptor family.

MOLECULAR CLONING AND GENE STRUCTURE

The genes for the human μ, d, and k opioid receptors have been isolated. The initial murine d opioid receptor cDNA was used by the authors to screen a human genomic phage library to identify clones containing the human genes. The authors subcloned μ, d, and k receptor fragments and sequence analysis of the three revealed the coding regions for the human opioid receptors. The human μ receptor gene shares 95 percent amino acid identity with the mouse

and rat μ receptors (Wang et al. 1994b). Three introns interrupt the μ receptor gene. The first two are located in the first and third intercellular domains. A third intron is located close to the carboxy tail of the receptor at amino acid position 397. Alternative splicing has been postulated at this site, which may result in a different carboxy terminus (Zimprich et al. 1994).

Analysis of the sequence of the human d gene fragment revealed 96-percent amino acid identity over 120 bases between the human genomic clone and the murine receptor cDNA (Selleri et al., in process). Knapp and coworkers (1994) also cloned a human d opioid receptor cDNA that encodes a 372 amino acid protein with 93 percent amino acid identity to the mouse d receptor. Partial sequence analysis by the authors of the human k gene showed a 100 percent DNA homology with the human sequence reported by Mansson and coworkers (1994). The introns in the murine k opioid receptor gene correspond to amino acid positions 86 and 184 (Yasuda et al. 1993). There appears to be conser-vation of these intronic positions in the human and rodent k, d, and μ opioid receptor genes (Mansson et al. 1994).

The opioid receptors, rhodopsin, the dopamine type 2 (D2)-like receptor, and the substance P receptors are a few among the many G-protein receptors reported to have introns within the coding region (Gingrich and Caron 1993; Hershey et al. 1991). In these receptors the intronic division within the coding region is similar, occurring at or near the membrane-spanning domain border. In each of the three opioid receptors the introns are found just beyond the first transmembrane spanning (TMS) region in the first intercellular loop and just beyond the fourth TMS region in the second cytoplasmic loop. The exception is the μ receptor, which also contains an additional intron near the carboxy terminus. The position of the introns in the human genes matches the position described for the rodent opioid receptors genes, which suggests stong evolutionary conservation of the intron/exon structure. The presence of introns and their precise location may be useful in defining functional units of the opioid receptors and in understanding the evolutionary divergence of the G-protein receptor genes.

Genetic regulation of opioid receptors is of great interest due to the role of the opioid system in syndromes believed to have heritable components such as pain response, metabolism of opiates, and addiction. Several groups of investigators are studying polymorphic markers for the opioid receptors. Two different types of

polymorphisms have been identified for the human μ receptor: a dinucleotide repeat and a restriction site polymorphism. PCR technology coupled with the identification of variable nucleotide repeats throughout the human genome has made it possible to rapidly type a particular allele. Only a fragment of the authors' clone of the μ receptor was found to contain a region containing a cytosine-adenosine (CA) dinucleotide repeat. PCR amplification of this region of DNA from 36 caucasian individuals reveals a significant degree of polymorphism (Selleri et al., in process). Wang and coworkers (1994b) also identified an Msp 1 polymorphism, which is under study to identify allelic variants associated with disorders of the opioid system.

CHROMOSOMAL LOCALIZATION OF OPIOID RECEPTORS

The chromosomal localization of the human μ, d, and k opioid receptor genes was determined by direct fluorescent in situ hybridization using the lambda phage DNA as probes to human metaphase chromosome preparations. Using this technique, the d receptor gene was mapped to chromosome 1p355-33, the k gene to chromosome 8q11.23-21, and the μ gene to chromosome 6q25-26 (Selleri et al., in process). Other investigators have also mapped the human opioid receptors to these locations (Befort et al. 1994; Wang et al. 1994b; Yasuda et al. 1994).

Each of the opioid receptors map to a single locus that apparently is not near receptors in which a mutation is suspected to cause any known human genetic disease. The chromosomal locations of the human μ, d, and k are in synteny with the reported position on mouse chromosomes (Selleri et al., in process). Southern analysis of murine opioid receptor genes in mouse neurogenetic mutants has not revealed gross alterations in the opioid receptor DNA (Befort et al. 1994; Kaufman et al. 1994). However, the information from polymorphic and chromosome markers will contribute to understanding the genetic regulation of the opioid receptors (Berrettini et al. 1994). Multiple familial and acquired diseases have been linked to abnormalities in G-protein receptors and the associated regulatory enzyme systems (Clapham 1993; Emala et al. 1994). Since the cloning of the opioid and other G-protein receptors has occurred quite recently, it is likely that additional diseases will soon be found that are linked to mutations or to regulatory alteration in these receptors.

LOCALIZATION OF OPIOID RECEPTOR mRNA

The field of opioid receptor biology has been advanced by anatomical studies of receptor distribution. Radiographic analysis with specific ligands revealed a unique neuroanatomical pattern for the μ, d, and k opioid receptors. With the cloning and isolation of the opioid receptors, direct analysis of the location of cells synthesizing opioid receptors can be made. A comparison of the correspondence between the binding data and the mRNA localization will provide insights into the trafficking and neural localization of these receptors.

The authors examined the distribution of the opioid receptor mRNA in the human prefrontal cortex and the striatum (Anton et al. 1994) and found that the patterns of distribution and expression level of the opioid receptor mRNA correlates well with the opioid receptor binding sites reported by autoradiography (Quirion and Pilapil 1991). In the prefrontal cortex, there is a high density of cells expressing μ opioid receptor mRNA found in cortical layers II and IV-V, and a moderate level of expression in layers I, III, and VI. Mu binding sites are the most selective for interaction with morphine and naloxone and they are also the most abundant and globally distributed opioid receptor in the human brain. The μ binding sites are concentrated in the superficial cortical laminae.

Delta opioid receptor mRNA was found in cells distributed homogeneously throughout all cortical laminae, although layers II-III and V-VI contained more densely labeled cells. Overall, the distribution of the d opioid receptor binding sites is similar to the μ receptor. In contrast, the k opioid receptor mRNA in the human prefrontal cortex is abundant in deep cortical laminae IV-VI. High levels of k opioid autoradiographic binding sites have also been reported within deeper cortical layers in this area, as well as other neocortical fields of human brain (Maurer et al. 1983; Pilapil et al. 1987; Quirion and Pilapil 1991). This localization in the deeper cortical layers has been postulated to induce the sedative properties of k agonists (Goodman and Snyder 1982).

The caudate-putamen is a central structure for the flow of information from the cortex to the ventral tegmental area and to the substantia nigra. Circuits involving these brain areas have been implicated in sensory-motor interaction, reward, and motivation. Moderate to high levels of hybridization signals were found in human caudate-putamen for μ, d, and k opioid receptor mRNAs (see table 1), although the density of μ opioid

TABLE 1. Opioid receptor mRNA distribution in human brain.

Region	Delta	Mu	Kappa
Prefrontal Cortex			
Layers			
I	0 to low	mod	0
II	mod	high	mod
III	high	low	mod
IV	mod	mod	high
V	high	high	high
VI	high	high	high
Striatum			
Caudate nucleus			
(interno-medial)	low to mod*	high*	mod to high**
Putamen	low to mod*	high*	high**

NOTE: 0, low, mod, and high indicate the relative density of cells expressing receptor mRNA.

KEY:0 = undetectable; mod = moderate; * = diffuse; ** = asymmetric cell clusters.

receptor was higher than for the d and k receptors. Cells containing µopioid receptor mRNA were homogeneously distributed throughout the human caudate-putamen. This homogeneous pattern of µ binding sites at the striatal level was also seen in human autoradiographic studies. These studies of the µ receptor highlight interesting species differences in the localization of opioid receptors. In the caudate-putamen, certain receptors and neurotransmitters preferentially bind the patch or matrix compart-ments. In the rat, guinea pig, and monkey the densest binding is observed for the µ receptor in the patches, and this neuroanatomical segregation is not seen in the human striatum (Herkenham and Pert 1981; Mansour et al. 1991).

Cells containing d and k opioid receptor mRNA were also found in the human caudate-putamen, though the levels of the d receptor were lower and more homogeneously distributed. A similar low density of d autographic binding sites was reported by Pilapil and coworkers (1987). Densely labeled k opioid receptor cells were grouped in clusters predomi-nantly in the caudate. These clusters of cells

expressing k opioid receptor mRNA suggests the patchlike striatal distribution. In support of these findings is the same striosomal compartmentalization of k opioid receptor binding sites in human caudate-putamen determined by the autoradio-graphic studies of Quirion and Pilapil (1991). To confirm the striosomal localization of k opioid receptor mRNA, additional histochemical studies are in progress using the combination acetylcholinesterase histochemistry and opioid receptor in situ hybridization in adjacent sections.

Prior to recent advances in the molecular characterization of the opioid receptors, the definition of classes of opioid receptors was made based on the order of receptor agonist and antagonist potency. The cloning of the opioid receptors has clearly identified a single gene for each of the human μ, d, and k opioid receptors. Conserved introns with the coding regions of these receptors may represent distinct functional domains. Conservation of the genomic structure suggests that there are overlapping functional and structural units, and perhaps evolution from a common ancestral gene. Conserved introns with the coding regions of these receptors may represent distinct functional domains. The question of subtypes remains, but the identification of an orphan opioid receptor promises interesting develop-ments on the horizon. The study of opioid receptor mRNA in the human establishes the foundation for further analysis of the precise distribution and mechanism of regulation of opioid receptor expression. Ideally, the analysis of the chromosomal localization will lead to the development of tools for the diagnosis and treatment of clinical syndromes associated with the opioid system.

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