Review Article

Mu opioid receptors in pain management

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A B S T R A C T

Most of the potent analgesics currently in use act through the mu opioid receptor. Although they are classified as mu opioids, clinical experience suggests differences among them. The relative potencies of the agents can vary from patient to patient, as well as the side-effect profiles. These observations, coupled with pharmacological approaches in preclinical models, led to the suggestion of multiple subtypes of mu receptors. The explosion in molecular biology has led to the identification of a single gene encoding mu opioid receptors. It now appears that this gene undergoes extensive splicing, in which a single gene can generate multiple proteins. Evidence now suggests that these splice variants may help explain the clinical variability in responses among patients.

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1. Introduction

Opiates have been used to manage pain for centuries. Opium, which contains a number of alkaloids including morphine and codeine, was eaten, smoked, and used as a tincture. After their isolation, morphine and codeine were used individually. Thousands of analogs have been generated in an effort to avoid some of the difficulties encountered with opiate use, such as respiratory depression, sedation, and constipation. Most of these agents act through mu opiate receptors, as defined by the selectivity of their binding for the three classes of opioid receptors that have been cloned. This is to be expected because the structure of morphine was used as a template for the synthesis of most of them. Clinicians have long observed that patients can have markedly varying responses to mu opioids. Some patients may encounter severe side effects such as nausea and vomiting with one agent, whereas having no difficulties at all with the other. For example, it is not uncommon to find patients with severe nausea/vomiting from morphine tolerating methadone without problems. Furthermore, the analgesic activity of the opioids also can vary among patients. One patient may find one mu opioid to be quite effective, whereas a different drug may not work anywhere near as well. However, the same drug may not be the best for all patients. This variability among patients has led to the accepted paradigm that analgesics need to be individualized. Opioid Rotation also illustrates differences among the mu opioids. In Opioid Rotation switching patients who are highly tolerant to one opioid to a different opioid often restores the analgesic effectiveness and sensitivity.1–3 While all mu drugs display tolerance with prolonged dosing, cross-tolerance is incomplete. That is, the tolerance to the second drug may not be as pronounced as the first, explaining why the switch is effective. All these features have raised questions about the mechanisms of these drugs, specifically how a single mu receptor could mediate these actions.

2. Early studies of mu opiate receptor and multiplicity

The first opioid receptor identified in binding assays in 19731–5 was the mu receptor. A receptor for the opiates had been predicted years earlier from a variety of traditional pharmacological approaches and the rigid structure activity studies of morphine and related chemical scaffolds.6–8 Because the studies used morphine-related probes, it is not surprising that the receptors were mu. However, several other families of opioid receptors have also been identified (Table 1). In vivo studies led Martin to propose the existence of kappa receptors, in addition to the mu receptors.9,10 Delta receptors, which are selective for the enkephalins, were identified11 after the isolation and identification of endogenous opioids.12–14 Indeed, it turns out that the endogenous ligand for the kappa receptors is another endogenous opioid, dynorphin A.15,16 Work is in progress developing new selective delta and kappa opioids for clinical use, but for the most part the drugs available clinically are
mu. There are several mixed agonists/antagonists that interact with both mu and kappa receptors, but they have limited use.

The first suggestion that there may be more than one class of mu receptors came from receptor binding studies in the mid 1970s that revealed a novel site with unusual characteristics. Although mu drugs like morphine labeled it with high potency, confirming its classification as a mu receptor, its ability to bind other drugs clearly distinguished it from the original mu receptor seen in receptor binding assays. The new site was termed mu1, whereas the original morphine-selective site was termed mu2. Understanding the pharmacological significance of these two mu receptors was facilitated by the development of selective antagonists that could selectively block the mu1 site and not the mu2 site. The naloxonazine-sensitive site corresponds to the mu1 site, whereas the naloxonazine-insensitive mu site is mu2. When given in vivo, these antagonists blocked morphine analgesia, but

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Drugs</th>
<th>Analgesia</th>
<th>Physical dependence</th>
<th>Dysphoria</th>
<th>Respiratory depression</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mu</td>
<td>Morphine</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>MOR-1</td>
</tr>
<tr>
<td>Kappa1</td>
<td>Dynorphin A USO, 488H</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>KOR-1</td>
</tr>
<tr>
<td>Kappa2</td>
<td>Ethylketocyclazocine</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>KOR-1/DOR-1</td>
</tr>
<tr>
<td>Kappa3</td>
<td>Naloxone benzoylhydrazine Levorphanol</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>See below</td>
</tr>
<tr>
<td>Delta</td>
<td>Enkephalin</td>
<td>Yes</td>
<td>No</td>
<td>7</td>
<td>No</td>
<td>DOR-1</td>
</tr>
</tbody>
</table>

Three major families of opioid receptors have been proposed. Subtypes of all have been proposed using pharmacological and molecular biological approaches. The genes for all have been established, except for kappa3. This subtype, which is important in the analgesic actions of a variety of opioid analgesics, including levorphanol and nalbuphine, is most likely a heterodimer involving one of the truncated 6TM MOR-1 splice variants and an unknown second receptor. However, this remains to be confirmed. DPDPE: [D-Pen2,D-Pen5]enkephalin.
not respiratory depression or the inhibition of gastrointestinal transit or many of the signs of physical dependence. Together, these studies implied that drugs such as morphine were acting through more than one mu receptor.

3. Molecular biology of mu opiate receptors

The mu receptors were first cloned in 1992 with the isolation of the delta receptor DOR-1 by two independent groups. This initial report confirmed the existence of the receptors within the G-protein coupled receptor family, one of the largest families of receptors known. Based on these structures, the other members of the mu receptor family were quickly cloned, including the mu and the kappa receptors. Soon after, yet another receptor was identified with a strong homology to the mu receptors but which was subsequently found to have a previously unknown peptide as its endogenous ligand, orphanan FQ/rocaine. The mu receptors are the most important class of receptors clinically. Most of the drugs used clinically act through these receptors and understanding their actions is important in defining their pharmacology. Pharmacological studies suggested more than one class of mu opiate receptor. This has now been confirmed at the molecular level. Although only a single mu receptor gene has been reported, the receptor undergoes extensive alternative splicing to generate a host of splice variants (Fig. 1).

After the cloning of MOR-1, much effort was placed on confirming its pharmacological relevance. The first studies involved using an antisense approach in which very short sequences of antisense targeted specific sequences of the mRNA, leading to its degradation. These initial reports quickly established that MOR-1 was critical in the production of morphine analgesia. However, the antisense approach can be used more extensively to perform antisense mapping to assess the presence of specific exons within the mRNA responsible for making a protein. Certain exon targets were important in morphine analgesia, including exons 1 and 4. However, antisense probes targeting exons 2 and 3 had little effect on morphine although they significantly diminished the activity of heroin and morphine-6β-glucuronide. Thus, at the molecular level there does appear to be receptor differences for these mu opiates.

The association of MOR-1 with morphine analgesia was further confirmed by the generation of several different knockout animals in which the MOR-1 gene was disrupted. In all of these different knockout animals, which targeted exons 1, 2, and/or 3, morphine lost all activity. However, in one exon 1 knockout mouse, heroin and M6G were still analgesic. This was quite confusing and it was initially suggested that these drugs were activating alternative opioid receptors, such as delta or kappa1. This possibility was eliminated with the finding that the residual heroin and morphine-6β-glucuronide analgesia remained in a triple knockout mouse, which had the same disruption of exon 1 of MOR-1 and additional disruptions of the delta (DOR-1) and kappa1 (KOR-1) receptors. What made this particular knockout mouse interesting was the continuous presence of a number of MOR-1 splice variants. Some of these, under the control of the exon 11 promoter, do not contain exon 1, perhaps explaining their continuous expression.

The splice variants associated with the mu opiate receptor can be separated into two categories based on their promoter. The gene encoding MOR-1 is quite complex with two distinct promoters. The primary one is associated with exon and generates a number of variants that are spliced at the 3' end in mice, rats, and humans. These are interesting from several perspectives. They are full length seven transmembrane domain G-protein coupled receptors. Structurally, they differ only at the very tip of the intracellular C-terminus. The initial opiate receptor cloned, MOR-1, contains 12 amino acids at the very tip of the C-terminus encoded by exon 4 (Fig. 2). In the other C-terminus spliced variants, these 12
Table 2
Opioid analgesia in MOR-1 knockout mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED$_{50}$ value (mg/kg, s.c.)</th>
<th>Exon 1 KO</th>
<th>Shift</th>
<th>Exon 11 KO</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td></td>
<td>WT KO</td>
<td></td>
<td>WT KO</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>&gt;100</td>
<td>1.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
<td>3.8</td>
<td>8.5</td>
<td>0.92</td>
<td>19.3</td>
</tr>
<tr>
<td>M6G</td>
<td></td>
<td>1.2</td>
<td>4.0</td>
<td>0.58</td>
<td>3.2</td>
</tr>
<tr>
<td>Fentanyl</td>
<td></td>
<td></td>
<td></td>
<td>0.024</td>
<td>0.230</td>
</tr>
</tbody>
</table>

WT and KO mice with a disruption of exon 1 of MOR-1 were tested with the indicated drug and their ED$_{50}$ values determined. Other groups of mice were generated with a disruption of exon 11. WT and KO mice were tested with the indicated drug and their ED$_{50}$ values determined. ED$_{50}$, median effective dose; WT, wildtype; KO, knockout.

4. The future

Pain management remains an art, dependent on the individualization of care. Not all patients can be managed with the same drugs. Indeed, the complexity of the clinical responses has long been evident. Many factors may play a role, but evidence is accumulating that much of this variability may result from the complexity of mu receptors. Early work from our laboratory proposed two subtypes, $m_{u1}$ and $m_{u2}$, based on selective antagonists. Studies showing differences between morphine and morphine-6-$\beta$-glucuronide further implied a third subtype. However, the molecular biology of the mu opioid receptor gene reveals the existence of dozens of mu receptor splice variants. Correlating these splice variants to the pharmacologically defined receptors has proven difficult. However, it seems likely that they play an important role in why patients respond so differently. As our understanding progresses, the critical question is whether or not we can use this knowledge to develop novel and useful analgesic lacking the detrimental actions of the currently available drugs.

Acknowledgments

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References

4. Simon EJ, Hiller JM, Edelman I. Stereoselective effects of opioids on mice with a disruption of exon 1 of MOR-1 were tested with the indicated drug and their ED$_{50}$ values determined. ED$_{50}$, median effective dose; WT, wildtype; KO, knockout. The detrimental actions of the currently available drugs.

The second set of variants is associated with exon 11, which is located approximately 30 kilobases upstream of exon 1. These variants include a number with quite unique structures. Unlike the exon 1-associated variants, which all encode full length, seven transmembrane domain receptors, many of the exon 11-associated variants generate truncated variants with only six transmembrane domains. Despite their unique structure, there is much evidence now to suggest that they are pharmacologically important. This derives from studies on mice in which all the exon 11-containing variants are lost because of disruption of this region of the gene (Table 2). Interestingly, morphine analgesia was not affected. However, the analgesic actions of heroin and the morphine metabolite morphine-6-$\beta$-glucuronide were significantly reduced. Looking at the drugs, it is apparent that the analgesic actions of mu opioids can be differentiated at the molecular level.


