

Insights into the genetics of mu-opioid analgesics: lessons from the clinic

Gavril W Pasternak, Head, Laboratory of Molecular Neuropharmacology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, USA

Over the past hundred years, a vast number of opioids have been developed and utilised by clinicians to treat pain. Thus, it is not surprising that clinicians have valuable insights into these drugs. Although many lessons have been learned, the most important is the need to individualise pain therapy among patients, both regarding the choice of drug and the dose. It is important to listen to the patient and treat the pain accordingly.

Most of the drugs used clinically are mu-opioid analgesics, being defined by their selectivity for the mu-class of opioid receptor. Yet these drugs are not all the same. Clinicians have long known that a patient may respond to one mu-opioid differently than to another. Side-effects may be problematic with one drug and not with another in one patient, while another patient may tolerate the first and not the second drug. Our inability to predict the responses of a specific patient has left the choice of a drug for an individual difficult, with the physician often sequentially trying several agents until one that works is found. It is this lack of predictability that often leaves clinicians uncertain as to how to treat pain and even questioning their patient's assessment of pain.

Opioid rotation also illustrates the differences among these opioids. When patients are highly tolerant to a drug and the dose can no longer be increased due to limiting side-effects, clinicians commonly switch the patient to an alternative drug and re-establish control of the pain. However, choosing the correct dose of the second opioid can be difficult. Studies over the past 50 years have established equianalgesic potency ratios for a wide range of these opioids. However, these equivalencies were established in naïve patients. Overall, they work quite well when starting a patient on an opioid. However, the ratios are quite different in the highly tolerant patient. Indeed, when switching a highly tolerant patient from one mu-opioid to another, it is common practice to calculate the equivalent dosage and then reduce this amount by 50–75%. While the mu-opioids demonstrate cross tolerance, it is often not complete, presumably explaining why lower doses of the second drug are effective.

How can these mu-opioids differ in so many respects and yet act through identical receptor mechanisms? This question has guided our laboratory research into the basic pharmacology of opioids for over two decades and led us to consider the possibility that there may be subtypes within the mu-opiate receptor family. Early binding studies performed back in the mid-1970s were suggestive of multiple binding sites.¹ Subsequently, our laboratory proposed two subcategories of mu-receptors in 1981, termed

mu₁ and mu₂.² Although both receptors bound morphine and other mu-opioids with high affinity, they differed in their affinity for other classes of opioids. Furthermore, mu₁ receptors were selectively blocked by the opioid antagonists naloxonazine and naloxazone. *In vivo*, naloxonazine selectivity blocked the analgesic effects of systemic morphine without influencing either respiratory depression or the inhibition of gastrointestinal transit, two potentially problematic side-effects of this classic drug.^{3–7} These observations suggested that there may be subtypes of mu-receptors and that these subtypes may mediate different morphine actions.

As noted earlier, clinicians have long known that some patients will be more responsive to one opiate agent than another. A similar situation exists in mice. Different strains of mice show varying sensitivities to morphine,^{8,9} the most prominent difference being the CXBK strain.¹⁰ Thus, there appears to be a genetic influence on opiate sensitivity. In addition, the sensitivities of various strains to different opiates vary independently. There are strains in which morphine is relatively inactive, whereas other mu-drugs, such as fentanyl, methadone, morphine-6β-glucuronide and heroin, are all effective analgesics.^{11,12} Thus, genetics supports the concept of mu-receptor multiplicity.

Opiate receptors were first cloned in 1992 with the isolation of a receptor for the delta opiate receptor.^{13,14} The mu-receptor was cloned in 1993 by several groups.^{15–18} When expressed in cells, the receptor showed all the characteristics anticipated of a mu-opioid receptor, binding mu-opiates with high affinity and showing poor affinity for delta and kappa opiates. Initial antisense studies in rats confirmed that this receptor was important for morphine analgesia.¹⁹ However, more detailed antisense mapping approaches raised the possibility that different mu-receptor subtypes mediate the actions of morphine and several other mu-opioids, such as heroin.²⁰ This concept was further supported by a knockout animal in which a portion of the MOR-1 gene was disrupted. Despite the complete loss of morphine actions, several other mu-drugs retained their analgesic actions, including heroin and morphine-6β-glucuronide.²¹ Clearly, these mu-drugs cannot be working through the same receptor.

In view of this evidence, our laboratory focused on isolating possible variants of the mu-receptor, in the hope that this would help us to explain some of the pharmacology. Two other groups had reported variants of MOR-1^{22,23} and we have now uncovered a multitude of new MOR-1 variants, each highly selective for mu-analgesics in receptor-binding

assays.²⁴⁻²⁶ Yet the regional distributions of these variants differed among themselves and from the original MOR-1 clone.²⁷⁻²⁹ This was particularly prominent in the dorsal horn of the spinal cord where it was found that MOR-1 and MOR-1C were both expressed, but in different cells. Furthermore, their distributions within a cell varied. Whereas MOR-1 itself was evenly distributed post- and presynaptically, the distribution of MOR-1C was almost exclusively presynaptic and only MOR-1C positive terminals co-localised with calcitonin gene-related peptide (CGRP), an important peptide involved in pain perception. Thus, at the molecular level we have now been able to identify multiple subtypes of mu-receptors differing in their regional and cellular distributions.

How might the existence of multiple mu-opioid receptor subtypes explain the clinical observations noted earlier? It is unlikely that the analgesic actions of any one opiate, such as morphine, are due to activation of a single receptor. It seems more likely that their analgesic actions represent the activation of a group of receptors. However, the group of mu-receptor subtypes activated by one opiate may not be identical to that of another opiate. While there may be some overlap among groups of mu-receptor subtypes activated by different drugs, it is not likely to be complete. This partial overlap of the receptor subtypes activated by different mu-opsiates may help to explain incomplete cross tolerance. It may also help to explain the variability in responses among patients, since the abundance of different receptor subtypes may be under independent genetic control and vary among patients. These possibilities raise interesting questions with regards the pharmacology of the mu-opsiates and the possibility of novel agents that lack significant side-effects. Foremost, however, these receptor subtypes illustrate the complexity of mu-opiate receptor systems and how it makes it impossible to predict which is the best drug for an individual patient and why clinicians need to continue to individualise the treatment of pain.

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Response

Ian Kitchen, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, UK

As Gavril Pasternak rightly points out, the interindividual responses to opioids seen in humans has long been recognised by clinicians. And this not only appears to reflect differences in pain control, which has often been the driver for opioid rotation, but also differences in the level and extent of the most troublesome side-effects of opioids, namely nausea, constipation and respiratory depression.

With such differences, it is easy to jump to the conclusion that the molecular explanation for this resides in the concept of receptor heterogeneity, and for over 20 years

Gavril Pasternak's group have been providing evidence for mu-receptor multiplicity. At times, it has been contentious. In the early days, most of the criticism about the concept of mu₁- and mu₂-receptors related to the perceived receptor activity of the pharmacological tools. There were many who felt that the behavioural effects of some of the compounds could be explained by actions at delta or kappa receptors and only with the advent of genetic techniques has the concept really progressed. One of the most persuasive pieces of evidence for distinct mu-receptor subtypes was provided by the studies with antisense oligonucleotides which were

able to clearly show very marked differences in responses to morphine and heroin. However, the use of antisense oligonucleotides is a pharmacological technique with limitations and has failed to be widely adopted. Yet the knockout mouse has afforded a very elegant and novel approach to study mu-receptor heterogeneity and the evidence from one strain clearly points to possible variants of the mu-receptor. However, others have not identified behavioural differences between mu-opioids in the mu-knockout mouse. The latest evidence for splice variants of the mu-receptor is most certainly important and again draws us back to this concept of mu-receptor heterogeneity. But whether these splice variants produce mu-receptor subtypes in a physiological environment is still to be determined.

So, if there are not mu-receptor subtypes, what are the other possibilities to explain the differences in responses to opioids at the clinical level? To my mind, there could be both alternative pharmacodynamic and pharmacokinetic explanations. At the pharmacodynamic level, it is possible that receptor dimerisation could explain the differences in

pharmacology. There is evidence in cell systems that opioid receptors heterodimerise, and the affinity of mu-agonists has been shown to differ markedly between monomer and dimer formations. At a pharmacokinetic level, there are very distinct differences particularly in relation to lipophilicity of compounds (for example, the polar compound morphine and the highly lipophilic compound fentanyl) and simple distribution differences may well account for the efficacy of drug rotation. In this regard, mu-analgesia can be manifested at peripheral, spinal and central levels, and the relative targets of opioids may well reflect their distribution at these sites rather than any differences in receptor affinities. Finally, a word of scepticism to stimulate still more debate. Even using gene knockout models to support the concept of mu-receptor heterogeneity, the conclusions from behavioural pharmacology still have some dependency on the selectivity of the compounds being used. Maybe it is still possible to explain differences in mu-agonist responses in animals and man by overlapping actions at delta or kappa receptors or indeed at non-opioid sites?