Opioids: first lessons from knockout mice

Brigitte L. Kieffer

Opioid receptors of the \( \mu \), \( \delta \) and \( \kappa \)-subtypes mediate the potent analgesic and addictive actions of opioid drugs. They also regulate responses to pain, stress and emotions when activated by endogenous opioid peptides. Recently, mice lacking opioid receptors or opioid peptides have been produced by gene targeting, providing molecular tools to study opioid function in vivo. Observations on mutant mice have shed new light on the mode of action of opioids, opioid receptor heterogeneity and interactions, and the involvement of each component of the opioid system in mouse physiology. In this article, Brigitte L. Kieffer reviews the first reported studies and discusses their therapeutic implications.

Opium has been extracted from poppy seeds (Papaver somniferum) for several thousand years to fight cough and diathesis, to relieve pain, and also because it evokes euphoria. The active ingredients of opium are alkaloid compounds, the so-called opioids. These molecules display strong analgesic and addictive properties and have been the subject of intense investigations. Morphine is the most active component of opium and was the first opioid to be isolated in the past century. Today, morphine remains the most potent pain killer used clinically, despite a considerable number of adverse side-effects. Although the 1980s and 1990s led to the development of many novel potent opioids by the pharmaceutical industry, the ideal analgesic is still awaited eagerly. The illegal abuse of heroin, a diacetylated morphine derivative, represents a major public health problem.

Several decades of pharmacology have shown that opioid alkaloids produce their biological action at the level of the CNS and specifically activate membrane receptors, thus interfering with a complex endogenous neurotransmitter system. The opioid system is composed of three receptor types known as \( \mu \), \( \delta \) and \( \kappa \)-receptors, which are activated by a family of structurally related endogenous peptides. Genes encoding three families of opioid peptides – pro-enkephalin, prodynorphin and pro-opiomelanocortin – and their receptors – \( \mu \)-opioid receptor, \( \delta \)-opioid receptor and \( \kappa \)-opioid receptor – have been cloned and characterized. This neuromodulatory system has been implicated in the control of behaviours that are essential for self and species survival, including responses to noxious information and stress, reward and motivation.

Opioid peptides and their receptors also control autonomic functions, including respiration, thermoregulation, and gastrointestinal motility, and they also modulate immune responses. Opioid receptors represent the primary targets for opioid drugs. Because of their high therapeutic potential for the treatment of severe pain, these compounds have spurred decades of pharmacological studies and the development of thousands of novel synthetic derivatives. Most efforts have aimed to increase the selectivity of opioid receptor agonists or antagonists towards \( \mu \), \( \delta \) or \( \kappa \)-receptors, with the hope that one could discriminate the biological events mediated by each receptor type and determine whether the desired and adverse effects of opioids could be dissociated. These studies showed that each receptor is distributed differently throughout the CNS (Ref. 19), with some regions expressing all three receptors (stratum and dorsal horn of the spinal cord), although not necessarily in the same neurones, and other regions exhibiting abundant sites for one receptor type only (thalamic nuclei for the \( \mu \)-receptor or the claustrum for the \( \kappa \)-receptor). They also revealed that each receptor is implicated in opioid function in a distinct manner and to a different extent.

Overall, it seems that \( \mu \)-receptor agonists display the best antinociceptive activity but also the highest abuse liability, that \( \delta \)-receptor agonists might exhibit less addictive potential, while being poor analgesic compounds and, finally, that the use of \( \kappa \)-receptor agonists for pain treatment should be restricted to the periphery because of the strong dysphoric properties of these compounds.

Until very recently, the issue of the contribution of each receptor type in opioid function was addressed by pharmacological approaches only, because agonists and antagonists were the only available tools. However, interpretation of the data is complicated by poor knowledge of the in vivo selectivity of opioid compounds, and their distinct metabolic or pharmacokinetic properties. Although binding affinities and selectivities of the compounds can be determined accurately in vitro using brain tissues or recombinant receptor preparations, one can never exclude the possibility that ligands described as highly \( \mu \)-, \( \delta \)- or \( \kappa \)-selective cross-interact with another receptor type in vivo. Their mode of action relies on the site of drug administration, the doses involved or the experimental paradigm (acute or chronic treatment). Thus, the exact contribution of each receptor in mediating the biological action of opioid drugs, or in regulating the endogenous opioid physiology, remains to be clarified by other approaches.

Molecular cloning of the receptors and spectacular advances in recombinant DNA methods now make it possible to address the issue by a genetic approach. The activity of known genes can be modulated in vivo using gene-targeting technology. Recently, mice lacking opioid peptides or receptors have been generated by...
homologous recombination. It is the purpose of this review to summarize what has been learnt from the very early analysis of these mutant mice, and to discuss the future outcome expected from the detailed observation of these exquisite 'tools'. Issues that are being addressed currently in several different laboratories are as follows: (1) How do opioids act at the molecular level, and what are the therapeutic implications? (2) What is the molecular target? (3) How crucial is the opioid system for survival and how are its various components implicated in responses to threatening environmental stimuli?

The mode of action of opioid drugs: which molecular target?

Morphine is the prototypic opioid. In contrast to many synthetic opioids that have been developed in the past 20 years, morphine is an opioid compound with low receptor selectivity. Binding studies performed on rodent brain membranes12,13 or recombinant receptor preparations14 have shown that morphine exhibits a preference for μ-receptors, with Kᵢ values in the nanomolar range, but also binds to δ- and κ-receptors with submicromolar affinities. This two-order-of-magnitude selectivity factor might be sufficient to examine μ-receptor responses using in vitro assays, but is rather low to ensure μ-receptor selectivity under in vitro testing conditions. Thus, it has long been believed that morphine activates multiple receptors in vivo. The investigation of morphine responses in mice lacking μ-receptors has now elucidated its molecular mode of action unequivocally (Fig. 1; Table 1; Refs 25–34). Mice that lack μ-receptors (MOR-deficient mice) have been generated by several laboratories, by disrupting exon 1 (Refs 25–27), exon 2 (Ref. 28) or exons 2 and 3 (Ref. 29). Analgesia, the main therapeutic action of morphine, was investigated extensively after acute subcutaneous (s.c.) administration of the drug. Morphine analgesia was abolished at doses that produce potent analgesia in wild-type mice (up to 50 μg kg⁻¹, s.c.) in tail immersion29, tail-dick25–27 and hotplate16–18 tests. Similar results were obtained following intrathecal or intracerebroventricular (i.c.v.) administration20. The injection of very high doses of morphine in mutant mice indicated a 110-fold increase in the ED₅₀ value when morphine was injected by the s.c. route, and no analgesia following i.c.v. administration (25 μg, that is, 15 times the ED₅₀ value in wild-type mice20). Altogether, these data show the absence of morphine antinociception in MOR-deficient mice at doses that classically induce strong analgesia in wild-type mice. This demonstrates that the MOR-encoded receptor is necessary to mediate morphine action on pain pathways and suggests that δ- and κ-receptors do not participate in morphine analgesia under standard experimental conditions. This is corroborated by the finding that morphine analgesia is preserved in mice deficient in the KOR (Ref. 30) or DOR (Ref. 31) genes.

Other major pharmacological actions of morphine have been studied in MOR-deficient mice. One of the most common adverse side-effects of morphine treatment is respiratory depression, a biological action that can be seen after acute administration and which requires tight control in the clinic. Matthes et al. showed that an analgesic dose of morphine (6 mg kg⁻¹, s.c.) decreased respiratory frequency and increased respiration time in wild-type mice. However, no change in respiratory parameters could be measured in similarly treated MOR-deficient mice20. Respiratory depression is the primary factor in the lethal toxicity of morphine. Loh et al. showed that an extremely high dose of morphine (1600 μg kg⁻¹, s.c.) was required to kill the mutant mice and that death occurred without any of the typical morphine effects20. Another undesirable action of morphine is constipation: Roy et al. demonstrated that a single s.c. injection of 15 mg kg⁻¹ morphine greatly inhibits gastrointestinal motility in wild-type mice, whereas no change in gastrointestinal transit was seen in mutant mice at doses up to 35 mg kg⁻¹ (Ref. 32). Acute morphine treatment also induces modification of locomotor activity. Tian et al. showed that horizontal locomotor hyperactivity and the inhibition of vertical locomotion, observed in wild-type mice, was absent in MOR-deficient mice20. Morphine also induces hyperphagia, a response that can be evaluated in animal models using the place-preference paradigm. Matthes et al. showed that morphine-

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**Fig. 1.** The molecular mechanism of action of morphine. Disruption of the μ-opioid receptor (MOR) gene leads to a complete loss of the main biological actions of morphine, demonstrating that both therapeutic and adverse effects of the prototypic opioid result from its interaction with a single gene product. Data from Refs 24–29, 30, 31.
conditioning (repeated low-dose injections, 3 mg kg\(^{-1}\), s.c.) did not induce place preference in mutant mice, under experimental conditions that greatly prolonged the time spent in the morphine-associated compartment in wild-type mice\(^2\). Chronic morphine treatment dramati-
cally modifies central as well as peripheral nervous sys-
tem physiology, including the development of physical
dependence, which is generally revealed by morphine
withdrawal symptoms. Matthes et al. showed the com-
plete absence of somatic signs of withdrawal (jumping,
sniffing, teeth chattering, ptosis, wet-dog shakes, paw
tremor, tremor and diarrhoea), and vegetative signs (weight
loss and hypothermia) in chronically morphine-
treated MOR-deficient mice, while all signs were readily
observable in wild-type mice\(^2\). In addition, the study
showed that upregulation of adenylate cyclase activity,
a well-described consequence of repeated morphine ad-
ministration, did not develop in the brain of mutant
mice. Therefore, the absence of \(\mu\)-receptors prevented
morphine withdrawal, both at the biochemical and
behavioural levels. Finally, another well-documented
consequence of chronic morphine treatment is im-
munosuppression. Gavériaux-Ruff et al. demonstrated
that repeated morphine administration induced lymph-
oid organ atrophy, reduced the ratio of immature
to mature thymic lymphocytes and greatly impaired
natural killer cell activity in wild-type mice. None
of these effects, however, could be observed in MOR-
deficient mice\(^3\).

In summary, all morphine responses investigated so
far are nullified in MOR-deficient mice. The genetic
approach clearly shows that the MOR gene product rep-
resents a mandatory molecular target for morphine. In
addition, the data show that the main biological actions
of morphine, both therapeutic and non-beneficial, are a
result of its interaction with the MOR gene product
exclusively. Therefore, it appears that activation of the
MOR receptor protein triggers a wide spectrum of bio-
logical events. A therapeutic implication of these find-
ings is that the development of novel drugs that would
specifically target this protein is unlikely to lead to the
ideal analgesic drug.

The detailed study of morphine responses in MOR-
deficient mice illustrates the usefulness of knockout mice
to clarify the molecular mode of action of opioid drugs.
The activity of classic opioid compounds that are being
used clinically or as pharmacological tools in academic
research can now be re-evaluated. Simonin et al. showed
that the antinociceptive action of the prototypic \(\kappa\)-recep-
tor agonist, U50488H, was abolished in KOR-deficient
mice, and that hypolocomotion and aversion produced
by the compound were strongly impaired in the mutant
mice\(^3\). These data establish a direct correlation between
the activity of the KOR gene product and the well-
documented \(\kappa\)-receptor pharmacology. However, it is
noteworthy that residual hypolocomotion is seen at high
U50488H doses (20 mg kg\(^{-1}\), s.c.) and some place aversion
can occur (with repeated administration of 1 mg kg\(^{-1}\),
s.c.) in mutant mice, observations that highlight the
limits of in vivo selectivity for an agonist which is
described as one of the best \(\kappa\)-selective agents. Using
MOR-deficient mice, Loh et al. demonstrated the absence

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DPDPE, cyclic[\(D\)-penicillamine\]2, \(D\)-penicillamine\]5-enkephalin; GI, gastrointestinal; M6G, morphine-6-glucuronide.
of analgesia following treatment with morphine-6-glucuronide, a morphine metabolite, and endomorphine 2, a recently reported, highly selective, endogenous \( \delta \)-receptor-selective peptide.\(^{25} \) Zhu et al. reported the abolition of spinal analgesia induced by cycloheximide, a protein synthesis inhibitor, and naltrindole, an \( \delta \)-selective antagonist, in DOR-deficient mice.\(^{25} \) In the future, mice lacking MOR, DOR or KOR genes will represent unique tools to identify unambiguously the molecular target of novel opioid drugs that are being developed for therapeutic purposes. Also, combinatorial double-mutant mice, expressing one receptor type only, will undoubtedly prove to be useful in this context.

**Opioid receptor heterogeneity and cross-talk: what do knockout mice tell us?**

A first level of complexity in opioid receptor pharmacology is the postulated existence of functional interactions between opioid receptors. A second consideration is the pharmacological diversity of opioid receptor sites, which exceeds that of the opioid receptor gene family. Although little has been done so far, the use of receptor-deficient mice has provided some insights into the molecular aspects of these highly debated issues.

The cross-talk between \( \mu \) - and \( \delta \)-receptors is best documented from the pharmacology, and is supported by the observation of analgesic responses to combinations of \( \mu \) - and \( \delta \)-receptor ligands or by computer analysis of binding data. The analysis of a ligand to \( \delta \)-receptor agonist responses in mice lacking \( \mu \)-receptors now provides a genetic means to determine whether \( \mu \) - and \( \delta \)-receptors act in a cooperative manner (Table 1). Three studies describe \( \delta \)-receptor-mediated analgesia in MOR-deficient mice,\(^{26,28,29} \) which exceed that of the opioid receptor gene family. Although little has been done so far, the use of receptor-deficient mice has provided some insights into the molecular aspects of these highly debated issues.

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Spontaneous activity of mutant mice (locomotion), their behaviour under threatening situations (acute pain and fear) and responses to opioid compounds have been evaluated using a large number of classically described behavioural tests. Locomotion was investigated in all mutant mice. This was done mainly by monitoring horizontal activity in a non-stressful environment using actimetry boxes, or under stressful conditions in an open field. Vertical locomotor activity is reflected by the number of boxes, or under stressful conditions in an open field. This was done mainly by monitoring horizontal activity using actimetry (i.e. in a non-stressful environment using actimetry). A main deficiency mice have also been examined using the rotarod test in a room with rotating wheels. Motor coordination skills have also been used to examine mice, reflecting the opioid peptides or their receptors. The tests are the open field, the elevated plus-maze or the elevated O-maze, where mice are exposed to an aversive environment. In these tests, exploration or the time spent in central areas (open field) or open sectors (plus- O-mazes) were monitored, and decreased activity in those parameters was considered to be an indication of increased anxiety. Aggressive behaviour was studied in mice lacking prepro-enkephalin using the resident-intruder test. In this model, mutant males were isolated for several weeks before another male was introduced into their cages. Tolerance to the intruder was then measured and increased aggression was revealed by reduced attack latencies and higher fighting scores.

Pain perception was evaluated by measuring the modification of nociceptive thresholds following a number of acute noxious stimuli. Thermal pain was examined in most mutant mice using the tail-flick or tail-immersion tests. Tail withdrawal from radiant heat (tail-flick) or hot water (tail immersion) reflects physiological events that occur mainly at the level of the spinal cord, and increased tail-withdrawal latencies are considered to be a measure of spinal analgesia. Thermal nociception was also investigated in the hot-plate test, where both paw licking and jumping latencies can be measured. Responses in this test involve effects that are integrated at higher brain levels, and decreased thresholds presumably reflect supraspinal analgesia. The two types of tests have been used to measure both basal nociceptive thresholds and opioid-induced analgesia. One model to evaluate inflammatory-tory pain is the formalin test, which was used for prepro-enkephalin- and -opioid receptor (KOR) deficient mice. This test, injection of formalin into the paw induces local and transient tissue damage, producing inescapable pain, which is quantified by measuring paw-licking, lifting or biting in the early phase of inflammation. Visceral pain was induced in both enkephalin- and KOR-deficient mice following intraperitoneal acetic acid injections and further measurement of abdominal contractions. In KOR-deficient mice, responses to mechanical pain were also examined in the tail-pressure test, where withdrawal latencies were measured following a locally applied increasing pressure. Stress-induced analgesia was evoked by forced swim in cold water, the water temperature determining the extent of opioid involvement in the analgesic response. Electric foot-shocks were also used to induce stress in the case of prepro-enkephalin-deficient mice. Post-stress levels of analgesia were evaluated by measuring nociceptive thresholds in tail-flick, hotplate or acetic acid writhing tests.

Commentary

The choice and design of experimental conditions need to be considered carefully when investigating the presence, or absence, of phenotypic changes in the knockout mice and, more specifically, when comparing results from different laboratories. As an example, it is reasonable to think that some apparent discrepancies in behaviours reported for the various MOR−/− mice (locomotion, pain thresholds and responses to b- receptor agonists) might result from different experimental testing conditions. Another factor of variability stems from distinct genetic backgrounds in the various mutant mouse strains. Classically, homologous recombination is performed in embryonic stem cells from various substrains of the inbred 129 mouse and chimera animals derived from the recombinant cells are backcrossed further on a more inbred strain resulting in hybrid mutant mice. Recipient strains are C57BL/6 for two reported MOR-deficient mice3,4, KOR-deficient mice3 and b-endorphin-deficient mice7. Another reported MOR-deficient mouse is a Swiss Black hybrid5 and mice lacking preproenkephalin are CD1 hybrids6. At present, this can obscure some of the conclusions because genetic background is known to influence many behaviours, in particular, responses to opioid analogues or nociceptive stimuli. The ideal situation would be to standardize backcrossing procedures and produce functionally related mutant mice with an identical genetic background, defined as most appropriate to study the relevant physiology. Although this is an achievable goal, it requires much effort. One must also consider that the several MOR-deficient mice reported to date all derive from different targeting constructs.

Finally, one should note that MOR-deficient mice arise from a targeted manipulation of the mouse genome and, in this respect, differ widely from the previously well-described, inbred, recombinant CXB; mouse strain obtained from the interbreeding of C57 and BALB/c mice. The latter mice exhibit low morphine-induced analgesia as well as other deficient antinociceptive responses, and display low b-receptor densities in some brain areas, genetic alterations that are responsible for those specific traits remain to be identified8.

References

Because DAMGO has been reported to recognize both $\mu_1$ and $\mu_2$ receptor sites with high affinity\(^3\), the data demonstrate that both $\mu_1$ and $\mu_2$ sites are encoded by the MOR gene. Zhu et al. described the absence of DPDPE ($\delta_1$), deltorphin II ($\delta_2$) and naltrindole ($\delta_1$ and $\delta_2$) in mice lacking the DOR gene, showing that $\delta_1$ and $\delta_2$ sites arise from the DOR gene\(^3\). Finally, Simonin and colleagues report the abolition of CI977 binding and U50488H biological activity in vivo in KOR-deficient mutant mice, indicating that $\kappa_1$ receptors, the best characterized $\kappa$-receptor binding sites, stem from the KOR gene\(^3\). The other described $\kappa$-receptor subtypes\(^3\) have been difficult to characterize because of the absence of specific ligands\(^3\), and the possibility that these sites arise from the three known genes is under investigation by both pharmacological\(^4\) and genetic means. Ultimately, disruption of the three genes simultaneously in a single animal will indicate if the cloned MOR, DOR and KOR genes encode the entire opioid receptor activity or whether other genes remain to be discovered.

The role of different components of the opioid system in mouse physiology

In addition to MOR-, DOR- and KOR-deficient mice, mutant mice lacking preproenkephalin\(^a\) and $\beta$-endorphin\(^b\) have been produced. Although the inactivation of all components of the opioid system has not been completed (prodynorphin-deficient mice have not been reported yet), the successful generation of homozygous mutant mice suggests that the absence of a single component of the opioid system is not lethal under homecage conditions. Furthermore, the adult mice are fertile and display no marked anatomical deficit, indicating that full activity of the endogenous system is not crucial during development. In the future, studies on combinatorial double- or triple-mutant mice might provide evidence about possible redundancy within the opioid system and its implication for survival. Autoradiographic mapping suggests that the lack of opioid receptors induces very few (MOR)\(^a\) or no (KOR)\(^b\) detectable compensatory changes in the expression of the remaining opioid receptor types. The expression of opioid peptide precursor genes also appears to be unchanged in receptor-deficient mice\(^b\). By contrast, the absence of preproenkephalin induced a marked upregulation of $\mu$-receptors in limbic areas\(^a\), showing regional-specific control of receptor expression by the peptide. Compensatory modifications in other functionally-related neurotransmitter systems have not yet been reported.

### Table 2. Mice lacking opioid receptors or peptides: behavioural alterations

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>MOR –/–</th>
<th>Refs</th>
<th>KOR –/– (Ref. 30)</th>
<th>Preproenkephalin –/– (Ref. 46)</th>
<th>$\beta$-endorphin –/– (Ref. 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>No change in H or reduction in vertical activity in OF</td>
<td>25</td>
<td>No change in H or OF</td>
<td>Reduction in horizontal and no change in vertical activity in OF</td>
<td>No change in H or OF</td>
</tr>
<tr>
<td>Basal nociception:</td>
<td>No change in Ti and HP</td>
<td>2B</td>
<td>No change in Ti and HP</td>
<td>No change in TF</td>
<td>Hyperalgesia in HP</td>
</tr>
<tr>
<td>Thermal pain</td>
<td>No change in Ti and HP</td>
<td>2B</td>
<td>No change in Ti and HP</td>
<td>No change in TF</td>
<td>Hyperalgesia in HP</td>
</tr>
<tr>
<td>Mechanical pain (TF)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Inflammation (I)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Visceral pain (W)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stress-induced analgesia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anxiety</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Others</td>
<td>Enhanced haematopoiesis</td>
<td>26</td>
<td>Larger litter size</td>
<td>Increased aggressive behaviour (II)</td>
<td>Increased body weight (10–15%)</td>
</tr>
<tr>
<td></td>
<td>Many other immune parameters unchanged</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduced sexual function in males</td>
<td>2B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F, formalin; H, horizontal locomotor activity measured in actimetry boxes; HP, hotplate; OF, open field; OM, elevated O-maze; PM, elevated plus-maze; R, rotarod; RI, resident–intruder; TF, tail-flick; TI, tail immersion; TP, tail pressure; V, vertical activity measured in actimetry boxes; W, acetic acid writhing.
Altersations of spontaneous behaviour in the mutant mice have been described in mice lacking μ or δ-receptors, as well as in opioid peptide-deficient mice (Table 2). Decreased horizontal locomotor activity was reported for mice lacking μ-receptors[8] and prepro-enkephalin gene[9], which suggests that a basal tone of both ligand and receptor modulate locomotion. It has been proposed that increased anxiety occurs in prepro-enkephalin-deficient mice[10], on the basis of their modified behaviour in the open-field and elevated O-maze tests. Mice that lack κ-receptors[11] do not appear to behave differently from their wild-type littermates in those animal models.

Thus, by studying MOR- and DOR-deficient mice, it can be determined whether the postulated anxiolytic effect of enkephalins is mediated by the μ-receptors or δ-receptors.

Responses to noxious stimuli and stressors both involve the opioid system. So far, pain perception has been investigated in acute nociceptive models only (Box 1, Table 2). Increased pain thresholds were observed following thermal stimulation in mice lacking μ-receptors[12] and prepro-enkephalin[13], suggesting a tonic involvement of these two components of the opioid system in the perception of thermal pain. This was not seen in mice lacking κ-receptors[14], which otherwise exhibited an enhanced sensitivity to visceral pain. Responses to stress have been investigated in mice lacking opioid peptides and the first evidence indicates an involvement of μ-endorphins[15], but not prepro-enkephalin[16], in the opioid component of stress-induced analgesia.

Other noticeable changes have been observed in mutant mice (Table 2). To date, however, too few studies have been reported to provide a clear picture of peptide-receptor partnerships in mouse opioid physiology. In addition, phenotypic changes observed in situations that recruit the endogenous opioid system rely on the behavioural paradigm that is used, or on the genetic background of the mutant mice, which influences basal tones and responsivity (Box 1). At present, it is difficult to correlate available data from mice lacking either a peptide or a receptor. Hopefully, in the future, mutant mice (Table 2) will provide a clear picture of peptide-receptor partnerships in mouse opioid physiology.

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Concluding remarks
Mice that lack opioid receptors have proven extremely useful to determine the molecular mode of action of a number of prototypic opioids and represent invaluable tools in drug discovery programmes for the development of novel therapeutic opioids. These mutant mice allow the clarification of some aspects of opioid receptor heterogeneity at the molecular level. Their use for the study of opioid receptor cross-talk is at an early stage, and the use of functional interactions of the opioid system with other neurotransmitters systems, including dopaminergic, glutamatergic or anti-opioid pathways[20], remains an entirely open field of investigation. The relevance of opioid receptor occupancy in behavioural and physiological responses might also be investigated by gene dosage effects in mono-allelic or bi-allelic mutant mice. Finally, the use of opioid receptor-deficient mice in sophisticated animal models for the study of chronic pain, stress and reward mechanisms should provide substantial insights into the tonic involvement of μ-, δ- or κ-opioid receptors under challenging environmental situations.

Selected references
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Kainate receptors: subunits, synaptic localization and function

Ramesh Chittajallu, Steven P. Braithwaite, Vernon R. J. Clarke and Jeremy M. Henley

Although it is well established that kainate receptors constitute an entirely separate group of proteins from AMPA receptors, their physiological functions remain unclear. The molecular cloning of subunits that form kainate receptors and the ability to study recombinant receptors is leading to an increased understanding of their functional properties. Furthermore, the development of kainate receptor-selective agonists and antagonists over the past few years is now allowing the physiological roles of these receptors and, in some cases, specific subunits to be investigated. As a consequence, the synaptic activation of postsynaptic kainate receptors and the presence of presynaptic kainate receptors that serve to regulate excitatory and inhibitory synaptic transmission have been described, and will be discussed in this article by Ramesh Chittajallu, Steven Braithwaite, Vernon Clarke and Jeremy Henley.

Since the discovery of multiple glutamate receptor subtypes, major advances in understanding the structures, distributions and roles that these receptors play in the CNS have been made. However, until recently, research into kainate receptors lagged behind that for AMPA and NMDA receptors owing to the lack of suitable pharmacological tools. The purpose of this review is to discuss how recent advances in molecular biology and pharmacology have led to the identification of the possible physiological roles of kainate receptors in the CNS.