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REVIEW

Analysis of opioid efficacy, tolerance, addiction and dependence from cell culture to human

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Opioid agonists are the most effective treatment for pain, but their use is limited by side effects, tolerance and fears of addiction and dependence. A major goal of opioid research is to develop agonists that have high analgesic efficacy and a low profile for side effects, tolerance, addiction and dependence. Unfortunately, there is a serious lack of experimental data comparing the degree to which different opioids produce these effects in humans. In contrast, a wide range of experimental techniques from heterologous expression systems to behaviour assessment in whole animals have been developed to study these problems. The objective of this review is to describe and evaluate these techniques as they are used to study opioid efficacy, tolerance, addiction and dependence.

LINKED ARTICLES
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Abbreviations
CNS, central nervous system; CPP, conditioned place preference; DAMGO, D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin; ERK1/2, extracellular signal regulated kinases 1 & 2; GIRK, G-protein coupled inward rectifying potassium channel; GTPγS, guanosine 5′-3′thio-triphosphate; HEK 293, human embryonic kidney 293 cells; Kv, voltage gated potassium channel

Introduction

Opioids are the most effective treatment for pain. Unfortunately, opioid use is limited by serious adverse effects such as respiratory depression, sedation and constipation. The development of tolerance, dependence and addiction during chronic opioid use further limit the clinical utility of these drugs. Opioid tolerance is characterized by a reduced responsiveness to an opioid agonist such as morphine and is usually manifest by the need to use increasing doses to achieve the desired effect. Clinically, more than 10-fold dose escalations of opioid dose in chronic pain management are common (Buntin-Mushock *et al.*, 2005) and yet, many studies show that relatively stable doses of opioids can provide pain relief for weeks or years (Eisenberg *et al.*, 2005; Farrar *et al.*, 2010). Addiction as defined by the compulsive, harmful use criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition is common among recreational opioid users, but relatively rare in chronic pain patients (Cowan *et al.*, 2001). Conservative estimates of patients prescribed long-term opioids who develop some sort of addictive disorder range from 2–6% (Fields, 2007) although both lower and higher rates have been reported (Ballantyne and LaForge, 2007; Noble *et al.*, 2010). The terms dependence and addiction are commonly used interchangeably, but the former will be used here in the context of the withdrawal syndrome that is characteristically observed on cessation of chronic opioid use or administration of opioid antagonists and the latter to models of compulsive drug use in animals and humans. Although animal studies have reported differences in analgesic efficacy, tolerance, addiction or withdrawal for different opioids, surprisingly few human studies have examined these

differences systematically. Although knowledge of genetic markers associated with opioid addiction and treatment are beginning to emerge (Yuferov *et al.*, 2010), the implications of these for effects of different opioids in humans remains unknown.

For more than a century, efforts to overcome the adverse effects of opioids have met with limited success. All opioids that produce analgesia also can cause tolerance, addiction and withdrawal, and all available opioids are misused (Hernandez and Nelson, 2010). Successful approaches to pharmacotherapy of opioid addiction continue to rely largely on substitution of short-acting agonists (heroin) often used dangerously (usually injection) with oral administration of long-acting high-efficacy agonists (methadone) or partial agonists (buprenorphine) (Lobmaier *et al.*, 2010). Nonetheless, a variety of animal and *in vitro* models provide a solid framework for translational research contributing to developments in opioid therapeutics that may reduce the severity of one or more of these adverse effects.

Long-acting opioid agonists have improved the management of ongoing pain and new formulations have improved the management of breakthrough pain (Inturrisi, 2002), but attempts to exploit different opioid receptor types have been less successful. Opioids that are more selective agonists for μ-, δ- or κ-receptors have had limited success because both the most potent desired actions (analgesia) and adverse effects, including dependence and addiction, are mediated by μ-opioid receptors (Kieffer and Gaveriaux-Ruff, 2002). Recent promising preclinical approaches to limit tolerance, dependence and addiction include simultaneous activation of more than one opioid receptor type (e.g. μ- and δ-receptors), selective targeting of heteromultimers, or μ-opioids that differentially activate distinct intracellular signalling cascades, particularly G-protein activation versus endocytosis (Berger and Whistler, 2010).

Efforts to better understand analgesic efficacy, tolerance, dependence and addiction as a way to enhance the analgesic effects and limit the liabilities have used a range of methodologies. The approaches include analyses at both the behavioural and cellular level in whole animals in addition to the use of reduced preparations such as heterologous cell systems and *in vitro* slice recordings. The type of information provided and the ability to generalize to clinical situations varies with the technique and issue. The present review will examine both behavioural and cellular approaches applied to four issues that limit opioid use in the treatment of clinical pain: limited antinociceptive efficacy, tolerance, addiction, and dependence.

Experimental approaches

Behavioural studies

Opioid antinociception, tolerance, dependence and addiction have been examined using a wide range of techniques in whole animals. The main advantage of using whole animals is that the nervous system is intact and opioid effects are linked to behaviour. A major problem, particularly with investigation of new drug candidates is that many studies fail to account for differences in pharmacokinetics and properties such as receptor efficacy. It is important to consider equivalence of drug concentration and exposure time of μ-opioid receptors (usually in brain) when comparing tolerance, addiction or dependence liability of different opioids using cellular or animal models, although this is rarely done. A range of different treatment schedules are commonly used to induce tolerance, dependence and addiction including repeated injections, osmotic minipumps, implantation of morphine base pellets (standardized morphine pellets can be obtained from the National Institute on Drug Abuse, USA) or sustained release emulsions. In addition, the physiological relevance of the species and behavioural assessment tools are quite variable and can be questioned. Pain sensitivity and antinociception have been shown to vary with mouse strain (Mogil *et al.*, 1999; Wilson *et al.*, 2003), and morphine potency varies with the nociceptive test (Morgan *et al.*, 2006b). Nonetheless, years of research have provided a range of methodologies to assess antinociceptive efficacy, tolerance, addiction and dependence.

Cellular studies

A vast amount of information about the cellular basis for opioid efficacy, tolerance, addiction and withdrawal has accumulated since the identification of genes encoding opioid receptors in the early 1990s (Chen *et al.*, 1993). Some of these data are derived by correlating cellular and behavioural changes produced by opioid administration in whole animals. This is a powerful approach in that it links cellular changes to specific behaviours such as antinociception or tolerance. These studies typically examine changes in the amount of a protein, but physiological changes can be studied in tissue taken from animals treated previously with opioids. Interpretation of the significance of these changes can be difficult. For example, the increase in extracellular signal regulated kinases 1 & 2 (ERK1/2) phosphorylation following chronic morphine administration has been interpreted as both contributing to (Narita *et al.*, 2002; Wilson *et al.*, 2003) and counteracting antinociceptive tolerance (Macey *et al.*, 2009).

In vitro *slice recordings*

Living tissue also can be removed from the nervous system to study neural responses *in vitro*. This approach provides a great deal of control of drug concentrations and a well-defined physiological end-point with the added benefit of having most connections to other cells intact. The most obvious deficits are the loss of afferent and efferent connections to the structure being studied and that mechanisms in one cell type may not be the same as another. In addition, the control that comes from isolating specific channels in specific neurons also prevents analysis of the normal function of the system. For example, analysis of the signalling cascade from μ-opioid receptors to voltage gated potassium (Kv) channels is known in great detail (Vaughan *et al.*, 1997), but isolation of these signalling pathways required chemically blocking currents in other channels and inputs from neurons containing glutamate and glycine. Moreover, the physiological relevance of opioid induced changes cannot be known. Opioid activation of Kv channels in periaqueductal gray neurons almost surely contribute to the antinociceptive effects of opioids, but

the relative importance of these channels over G-protein coupled inward rectifying potassium (GIRK) or Ca channels or modulation of glutaminergic inputs cannot be determined using *in vitro* techniques alone. Nonetheless, *in vitro* slice recordings have provided a depth of understanding of opioid signalling pathways that would not have been possible with other approaches.

The effects of withdrawal of opioid drugs on cellular properties of tissues taken from animals after chronic treatment with opioids also have been studied (Williams *et al.*, 2001; Christie, 2008). Excessive increases in action potential or synaptic activity during spontaneous or antagonist precipitated withdrawal in neurons and systems usually inhibited by opioid agonists appear to reflect dependence at the cellular level but are not necessarily causally connected with dependence in the behaving animal or human.

Natural cells in culture

Naturally occurring neurons can be dissociated and maintained in culture. This is a common approach to study the effects of opioids on neurons from the dorsal root ganglion (Werz and Macdonald, 1982), but can also be used with neurons in the brain and spinal cord. The advantage of this approach is the control that comes with an isolated preparation and the fact that these are naturally occurring neurons with all of the signalling proteins that normally exist. The problem is that removal of the neurons damages the cells and deprives them of the connections, growth factors and other chemicals in their natural environment. For example, microglial activation appears to contribute to opioid tolerance (Zhou *et al.*, 2010) but the influence of opioids on neuronal-astroglial and microglial interactions *in vivo* remain poorly understood.

Heterologous expression systems

The ability to maintain healthy cells in culture and express proteins in those cells has greatly enhanced the analysis of opioid mechanisms of action. The main advantage of this technique is control. The function of specific proteins can be studied by inserting a protein or the genetic material necessary to make the protein into a cell with known characteristics. This approach is particularly useful to determine the electrophysiological or biochemical properties of a protein (Tate and Grisshammer, 1996). The problem is that the physiological relevance of these 'Frankenstein' cells is unknown and results obtained for opioid receptors expressed in one cell line might not generalize to other cell systems, native neurons or to the behaving animal. For example, opioids that produce maximal stimulation of GTPγS in Chinese hamster ovary cells transfected with μ-opioid receptors do not necessarily show the same relative stimulation when applied to the membranes of thalamic neurons (Selley *et al.*, 1998).

The amount of protein and the presence or absence of various signalling pathways will influence opioid effects. Although over-expression enhances the ability to examine protein biochemistry, protein behaviour and stoichiometry of other signalling proteins can change depending on the level of receptor expression (Law *et al.*, 2000). Nonetheless, Xenopus oocytes, HEK 293 cells, and other cell types have been used effectively to examine opioid interactions with μ-opioid receptors.

Analgesic efficacy

Efficacy, as the term is used here, refers to receptor signalling efficacy, or the magnitude of a receptor-mediated effect produced by a drug relative to receptor occupancy (Clarke and Bond, 1998; Strange, 2008). Relative efficacies of a series of agonists can be estimated readily if they behave as partial agonists (maximum response is less than that achieved with a full agonist) which should be confirmed by demonstrating partial antagonism of a full agonist by the drug in question. This is often not feasible for opioids *in vivo* and partial irreversible antagonism of an appropriate fraction of μ-opioid receptors is required to estimate relative efficacies (Kumar *et al.*, 2008). A goal of opioid research is to develop compounds with high analgesic efficacy because these are less affected by tolerance development in animal studies than low-efficacy agonists (see below). However, low-efficacy agonists can have other advantages, for example, the low-efficacy opioid, buprenorphine, produces less respiratory depression and is therefore safer in humans than high-efficacy agonists (Dahan *et al.*, 2006)

Opioid efficacy *in vivo* can be measured using a wide range of techniques and endpoints in addition to analgesia. Given that most measures of efficacy can vary depending on the endpoint, particularly when measuring maximal responses to drugs (Clarke and Bond, 1998; Strange, 2008), an important question is whether analgesic efficacy correlates as well as it should with direct μ-opioid receptor signalling efficacy for endpoints measured using *in vitro* techniques. This section will review the different approaches used to evaluate opioid efficacy and consider some of the technical limitations of different approaches.

Human studies

With the exception of long-established evidence that buprenorphine is a low-efficacy opioid (Jasinski *et al.*, 1978), no large-scale randomized controlled trials examining the analgesic efficacy of different opioids have been conducted in humans. Part of the problem is that large-scale clinical trials to assess relative efficacy are difficult to conduct from both a practical and ethical standpoint. In addition, analgesic efficacy can vary with the type of pain (cancer, neuropathic, surgical), method of opioid administration (oral, intravenous, transdermal), and age and sex of the patient. The six most common clinically used opioids are morphine, oxycodone, hydromorphone, fentanyl, buprenorphine and methadone (Pergolizzi *et al.*, 2008). The widespread use of these opioids indicate that they are effective in treating pain. Experimental data indicate that these drugs are effective in treating pain even for chronic conditions such as neuropathic pain (Eisenberg *et al.*, 2005; Farrar *et al.*, 2010). However, with few exceptions the relative analgesic efficacy of opioids is not yet known in humans and the implications of this for tolerance, addiction and dependence are not well established. In contrast, the antinociceptive efficacy of opioids has been well

studied in experimental animals and it is reasonable to expect that these findings might extrapolate to humans.

Behavioural studies in experimental animals

A wide range of nociceptive tests has been developed to assess nociception in laboratory animals (Le Bars *et al.*, 2001). Rats and mice are by far the most common species used in studies assessing the antinociceptive effects of opioids. Although there are a number of studies that analyse antinociceptive efficacy specifically, it is not known if relative efficacy is consistent across nociceptive tests. These tests vary in the type of stimulus (thermal, mechanical and chemical), duration/severity of pain (acute vs. neuropathic or inflammatory) and types of response (supraspinally organized response vs. reflexive). Given that the antinociceptive potency of morphine varies with nociceptive test (Morgan *et al.*, 2006b), it is likely that morphine efficacy also varies. A more difficult question to answer is whether the relative efficacy of different opioids varies depending on the nociceptive test.

Efficacy is also difficult to assess because most nociceptive tests impose an artificial definition of antinociception to limit potential damage to the animal. For example, the two most common nociceptive tests, the tail flick and hot plate tests, typically define antinociception as a latency of 10 and 50 s respectively. Given that all clinically used opioids will increase a rodent's tail flick latency to 10 s if the dose is high enough, relative efficacy is determined by measuring the antinociceptive effect of an opioid following pretreatment with an irreversible antagonist to reduce the number of μ-opioid receptors. High-efficacy agonists require few μ-opioid receptors to produce antinociception whereas irreversible block of a subset of μ-opioid receptors will reduce the maximal antinociceptive effect of low-efficacy agonists.

The most complete analysis of antinociceptive efficacy to systemically administered opioids has been conducted by Yoburn and colleagues using partial irreversible antagonism to estimate relative efficacy (tau). These studies assessed nociception with the tail flick test in mice and showed that fentanyl has the greatest relative efficacy (tau = 58), followed by etorphine (52), methadone and morphine (39), hydromorphone (35), oxycodone (20) and hydrocodone (18) (Kumar *et al.*, 2008; Sirohi *et al.*, 2008; Madia *et al.*, 2009). Other studies with rats show similar results: greater antinociceptive efficacy for fentanyl, etorphine and methadone than morphine (Adams *et al.*, 1990; Walker *et al.*, 1998) (Table 1). Additional studies examining relative antinociceptive efficacy using chronic pain tests would help link these findings to humans where opioids are primarily used to treat chronic conditions.

If these animal data are comparable to human, then the six most commonly used opioids to treat pain in humans include both high-efficacy (fentanyl) and relatively low-efficacy (oxycodone) agonists. This range of opioid efficacies indicates that factors other than efficacy are more important in determining opioid use in humans than relative efficacy. These factors include side effect profile, onset of action, drug interactions, abuse potential, cost and type of pain (Pergolizzi *et al.*, 2008). The importance of these factors is not surprising given that the overall analgesic efficacy of opioids is quite good compared with most other treatments. Of course, it is possible that the antinociceptive efficacy of opioids in humans and rodents differ.

Table 1

Relative antinociceptive efficacy of opioids in whole animal studies

Rat tail flick studies:	
Walker *et al.* (1998)	Etorphine = etonitazene > morphine > buprenorphine
Adams *et al.* (1990)	Fentanyl > methadone > morphine = levorphanol
Mouse tail flick studies:	
Goode and Raffa (1997)	Sufentanil > DAMGO > morphine
Pawar *et al.* (2007)	Etorphine (tau = 52) > morphine (39) > oxycodone (20)
Sirohi *et al.* (2008)	Fentanyl (tau = 58)
Kumar *et al.* (2008)	Hydromorphone (tau = 35)
Madia *et al.* (2009)	Methadone (tau = 39) > hydrocodone (tau = 18)

The fact that nociception is assessed differently in rodents and pain patients may contribute to a difference in measured efficacy. For example, antinociceptive efficacy on a nociceptive reflex (i.e. the tail flick test) may not be comparable to the antinociceptive efficacy of opioids to inhibit a supraspinal sensation such as pain. Supraspinally organized nociceptive tests such as the hot plate are influenced by a range of non-nociceptive stimuli such as body weight and habituation (Gunn *et al.*, 2010). These factors have less of an influence on reflexes suggesting that nociceptive reflexes may be a good way to isolate the antinociceptive effects of opioids. However, opioid efficacy appears to vary depending on whether A-delta or C-fibres are activated by the stimulus (McCormack *et al.*, 1998). Nonetheless, opioids that are effective in the clinic, such as morphine and fentanyl, produce very good antinociceptive effects in rats tested with the hot plate or tail withdrawal (Morgan *et al.*, 2006b).

Other factors such as sex and chronic pain also alter the antinociceptive effects of opioids. μ-Opioid receptor agonists tend to produce greater antinociception in males compared with females in both humans and other animals (Craft, 2008; Fillingim *et al.*, 2009), and the magnitude of this difference is inversely related to agonist efficacy. That is, low-efficacy μ-opioid receptor agonists produce greater sex differences than high-efficacy agonists (Cook *et al.*, 2000; Craft *et al.*, 2001; Terner *et al.*, 2003). Changes in opioid antinociception caused by chronic pain are complicated by the fact that baseline pain sensitivity is enhanced (Przewlocki and Przewlocka, 2001). Less clear is whether sex and chronic pain alter the relative efficacy of opioids in humans.

Cell studies

Opioids activate a wide-range of signalling cascades upon binding to μ-opioid receptors. Some of these signalling pathways such as those linked to GIRK, Kv^{+} and Ca^{++} channels are

Table 2

Relative efficacy to induce μ-opioid receptor internalization

Tissue	MOPr internalization	No MOPr internalization	Citation
Oocytes	DAMGO, methadone, fentanyl	Morphine	Celver *et al.* (2004)
HEK cells	B-endorphin, DAMGO, etorphine*	Codeine, heroin, buprenorphine, morphine	Keith *et al.* (1998)
HEK cells	DAMGO, etorphine, methadone	Morphine, buprenorphine	Whistler *et al.* (1999)
HEK cells	DAMGO, sufentanil, etonitazene*	Oxycodone, morphine, buprenorphine	Koch *et al.* (2005)
HEK cells	Etorphine > fentanyl	Morphine > buprenorphine	Zaki *et al.* (2000)
HEK cells	Fentanyl, methadone, oxycodone	Morphine, buprenorphine	Melief *et al.* (2010)
AtT20 cells	DAMGO > methadone	Morphine, pentazocine	Borgland *et al.* (2003)
LC slice	ME, etorphine, methadone	Morphine, oxycodone	Arttamangkul *et al.* (2008)
Rat cord	DAMGO, endomorphin, remifentanil	Morphine	Trafton *et al.* (2000)
HEK cells	Rank order: etorphine > DAMGO = methadone > fentanyl > morphine > buprenorphine		McPherson *et al.* (2010)

*Fentanyl and methadone produce partial MOPr internalization.
DAMGO, D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin; HEK, human embryonic kidney cells; LC, locus coeruleus; ME, met-enkephalin; MOPr, μ-opioid receptor.

activated regardless of the opioid (Christie *et al.*, 2000), whereas other pathways are selective depending on the opioid. For example, met-enkephalin, etorphine, methadone, morphine, oxymorphone and oxycodone all produce maximum activation of GIRK channels, but of these opioids only met-enkephalin, etorphine and methadone produce significant levels of μ-opioid receptor internalization (Arttamangkul *et al.*, 2008). The ability of high-, but not low-efficacy μ-opioid receptor agonists to produce efficient μ-opioid receptor internalization occurs regardless of research technique (Table 2). One exception is morphine induced μ-opioid receptor internalization in dissociated striatal cells (Haberstock-Debic *et al.*, 2005). In the most comprehensive survey of relative efficacy of opioids in heterologous cell expression systems to date, morphine-signalling efficacy (GTPγS binding) correlated well with other opioids for receptor phosphorylation, arrestin recruitment and μ-opioid receptor internalization (McPherson *et al.*, 2010). A full understanding of why some μ-opioid receptor agonists induce receptor phosphorylation and internalization and others, such as morphine, does not have yet to be resolved.

Comparison of opioid efficacy using other endpoints and tissue preparations has been reported. Although different opioids are used in different experiments, efficacy for these endpoints is relatively consistent with antinociceptive efficacy as the examples in Table 3 demonstrate. The main exception is that morphine efficacy can vary widely depending on the signalling pathway. For example, morphine produces good inhibition of adenylyl cyclase (Keith *et al.*, 1998; Koch *et al.*, 2005), but produces no phosphorylation of ERK1/2 as occurs following fentanyl administration (Macey *et al.*, 2006). These differences may be related to the limited ability of morphine to induce μ-opioid receptor internalization and the signalling cascades linked to that process. The behavioural significance of some of these signalling cascades is uncertain because opioids activate a wide range of signalling pathways, but only a subset of these are known to contribute to the antinociceptive effects (Mitrovic *et al.*, 2003).

Tolerance

The operational definition of tolerance is a decrease in effect following repeated or prolonged administration of a specific dose. It is important to distinguish short-term or 'acute' tolerance that develops within minutes to several hours (Mathews *et al.*, 2008) from long-term tolerance that develops during prolonged exposure to opioids. The former is probably more closely related to mechanisms of rapid μ-opioid receptor desensitization and trafficking than the latter. Experimentally, tolerance is best demonstrated by a rightward shift in the agonist dose-response curve after repeated administration for days or weeks. This decrease in effect can be caused by pharmacokinetic, pharmacodynamic or conditioning mechanisms. Studies claiming to demonstrate that a novel opioid produces less tolerance than conventional opioid analgesics should establish that pharmacokinetic considerations are not relevant. For example, tolerance is certainly related to duration of exposure of μ-opioid receptors to elevated opioid concentrations, with continuous exposure producing greater tolerance than intermittent exposure (Duttaroy and Yoburn, 1995). Few studies measure concentrations of the drug at the relevant target (CNS) or control for duration of action of each dose. CNS concentration is less of a concern in animal studies in which direct opioid administration into specific parts of the CNS such as the periaqueductal gray (Morgan *et al.*, 2006a) or spinal cord (Stevens and Yaksh, 1989) produce tolerance. Although repeated opioid administration produces some differences in neural adaptations from continuous administration (Christie, 2008), unambiguous comparison of tolerance development with different agonists requires steady state treatment (e.g. minipumps) with agonists that achieve equivalent effects in the CNS to ensure equivalent levels and durations of receptor stimulation. This is rarely achieved.

Despite these limitations, the measurement of tolerance is strongly influenced by opioid efficacy with high-efficacy agonists displaying less obvious tolerance than low-efficacy

Table 3

Relative efficacy to alter μ-opioid receptor signalling

Tissue preparation	Rank order of efficacy	Citation
Inhibition of cAMP in HEK cells	Etorphine > DAMGO > morphine	Keith *et al.* (1998)
Inhibition of cAMP in HEK cells	Etonitazene > DAMGO = morphine > methadone > fentanyl > buprenorphine	Koch *et al.* (2005)
Activation of GIRK in HEK cells	DAMGO ≥ morphine > methadone	Whistler *et al.* (1999)
Arrestin recruitment in HEK cells	DAMGO = etorphine > methadone = fentanyl > morphine	McPherson *et al.* (2010)
GTPγS binding in HEK cells	DAMGO > methadone > fentanyl > etorphine > morphine > buprenorphine	McPherson *et al.* (2010)
GTPγS binding in SH-SY5Y cells	DAMGO = fentanyl > morphine > buprenorphine > pentazocine > levallorphan	Traynor and Nahorski (1995)
GTPγS binding In HEK cells	Etorphine > morphine > fentanyl > buprenorphine	Zaki *et al.* (2000)
cAMP inhibition in HEK cells	Etorphine = morphine = fentanyl > buprenorphine	Zaki *et al.* (2000)
$G_{\alpha i1}$ binding in HEK cells	DAMGO > methadone > fentanyl = morphine > buprenorphine	Saidak *et al.* (2006)
$G_{\alpha oA}$ binding in HEK cells	DAMGO > morphine ≥ methadone > fentanyl > buprenorphine	Saidak *et al.* (2006)
GIRK currents in oocytes	Etorphine > sufentanil > DAMGO > morphine > methadone > buprenorphine	Yu *et al.* (1997)
Ca^{2+} currents in AtT20 cells	DAMGO ≥ methadone > morphine > pentazocine	Borgland *et al.* (2003)
ERK1/2 phosphorylation in striatal culture	Fentanyl >> morphine	Macey *et al.* (2006)

DAMGO, D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin; ERK1/2, extracellular signal regulated kinases 1 & 2; GIRK, G-protein coupled inward rectifying potassium; HEK, human embryonic kidney cells.

agonists. This relationship is well established in both behaving animals (Stevens and Yaksh, 1989) and isolated cells (Christie *et al.*, 1987), and arises in part because highly efficacious opioids produce maximal effect with low receptor occupancy. Receptor signalling efficacy of both the treating and challenge opioids also influences the magnitude of tolerance, as well as efficacy for different downstream signalling cascades (Berger and Whistler, 2010). Continuous administration of low- compared with high-efficacy agonists produce greater tolerance to challenge agonists regardless of the efficacy of the latter (Madia *et al.*, 2009).

A major goal is to determine the mechanisms underlying tolerance so treatments that block these mechanisms can be developed to maintain analgesia for long periods of time. The problem with these studies is that numerous mechanisms for tolerance have been proposed (Christie, 2008), but no integrated theory exists. Tolerance could be caused by molecular adaptations in neurons with μ-opioid receptors, changes in interactions between cells, activation of an independent oppositional system as could occur with opioid-induced hyperalgesia (Zeng *et al.*, 2006; Vera-Portocarrero *et al.*, 2007), or some combination of all of these. Additional research linking molecular changes to behaviour is needed to clarify the mechanism for tolerance to opioids.

Human studies

Dose escalation in human pain patients receiving chronic opioid therapy is well established (Collett, 1998; Tobias, 2000), although many patients maintain a relatively consistent dose for months once a therapeutic dose is achieved (Cowan *et al.*, 2001; Farrar *et al.*, 2010). Dose escalation is a common occurrence for illicit opioid users (Cowan *et al.*, 2001), and anecdotal reports of complete tolerance to doses of morphine up to approximately 500-fold the normal dose in single infusions in human addicts have been documented (Jaffe, 1985). The magnitude of tolerance is more difficult to ascertain in chronic pain patients. Many patients stop taking opioids because of adverse effects or ineffective pain relief (Noble *et al.*, 2010). Tolerance to the analgesic effects likely contributes to this decrease. A decrease in analgesia can also be caused by factors unrelated to tolerance such as an increase in pain related to disease progression (Portenoy, 1994). Tolerance also appears to be limited when opioids are self-administered (Hill *et al.*, 1990). Thus, although tolerance to the analgesic effects of opioids has been demonstrated in pain patients, the magnitude of the problem is debatable (Collett, 1998; Tobias, 2000).

Although whole animal and cellular studies would predict that low-efficacy agonists should show greater tolerance than high-efficacy opioids (see below), there is no evidence for this in the limited studies that have been performed. When directly comparing tolerance development during continuous administration of transdermal fentanyl (high-efficacy) versus buprenorphine (low-efficacy) (Sittl *et al.*, 2006) greater tolerance was found for fentanyl. The basis for this discrepancy with animal studies is uncertain but could be due to internalization and down-regulation of μ-opioid receptors using agonists such as fentanyl (Patel *et al.*, 2002).

Behavioural studies in experimental animals

Tolerance to the antinociceptive effects of opioids in animal experiments is well documented. Tolerance has been shown with as few as a single injection (Cochin and Kornetsky, 1964; Melief *et al.*, 2010) and in various parts of the nervous system including the periaqueductal gray (Morgan *et al.*, 2006a), spinal cord (Stevens *et al.*, 1988) and periphery (Aley and

Levine, 1997). Neither the mechanism underlying tolerance nor the degree to which tolerance develops to different opioids at each of these sites is necessarily the same. Indeed, at the cellular level, recent findings indicate that the mechanism underlying tolerance may differ depending on the opioid. Specifically, interaction of the μ-opioid receptor with G-protein receptor kinase appears to contribute to tolerance to high-efficacy μ-opioid receptor agonists such as D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin (DAMGO), but not lower-efficacy agonists such as morphine (Hull *et al.*, 2010; Melief *et al.*, 2010), although these studies examined only the short-term tolerance that may be more closely related to mechanisms of rapid receptor desensitization than long-term tolerance.

Tolerance appears to develop to all opioids, but the magnitude of tolerance varies depending on the route of administration and agonist efficacy. Repeated injections of morphine, fentanyl, etorphine, oxycodone and meperidine in mice produce comparable rightward shifts in the dose–response curve of the same agonist for antinociception, but continuous administration of these drugs produces much greater cross-tolerance to morphine than etorphine or fentanyl (Duttaroy and Yoburn, 1995). Continuous intrathecal infusion of morphine also produces greater tolerance than infusion of DAMGO or fentanyl (Stevens and Yaksh, 1989) indicating, as expected, that high-efficacy agonists show less tolerance than lower-efficacy agonists.

Low-efficacy μ-opioid receptor agonists are also more susceptible to cross-tolerance regardless of whether animals are pretreated with high- or low-efficacy agonists (Sosnowski and Yaksh, 1990; Barrett *et al.*, 2001; Walker and Young, 2001). In fact, rats show a greater shift in the morphine than the sufentanyl dose–response curve even when pretreated with sufentanyl to induce tolerance (Sosnowski and Yaksh, 1990). The opposite also appears to be true. Induction of tolerance with low- compared with high-efficacy μ-opioid receptor agonists produces greater cross-tolerance to morphine (Pawar *et al.*, 2007; Kumar *et al.*, 2008). However, this relationship between efficacy and cross-tolerance to morphine is only evident with continuous, not repeated administration of the pretreatment opioid (Madia *et al.*, 2009). This difference might not be caused by underlying adaptations (tolerance) at the μ-opioid receptor, but may reflect high levels of receptor occupancy by low-efficacy agonists such as morphine. These issues can only be properly resolved by directly determining receptor tolerance produced by different agonists in tissue from treated animals.

Cell studies

Tolerance can be studied at the cellular level by either correlating chronic opioid administration with changes in neural signalling or by measuring changes in cell activity with prolonged application of an opioid. A surprisingly large number of cellular changes have been reported following chronic morphine administration that can influence tolerance either directly at the μ-opioid receptor or via downstream effects on excitability (Christie, 2008). Analysis of data for all downstream mechanisms is too large an undertaking for this review especially given that few of these studies have compared the effects of different opioids. In general, when endpoints such as GTPγS binding or G-protein βγ subunit activation of ion channels that closely reflect μ-opioid receptor function have been examined, loss of function or uncoupling of receptors is consistently observed (Williams *et al.*, 2001; Christie, 2008).

A number of studies have examined the ability of different opioids to induce μ-opioid receptor desensitization as a measure of tolerance. Desensitization is typically measured as a decrease in K^+ or Ca^{2+} currents that occur during continuous opioid exposure. This rapid loss of receptor sensitivity occurs much more quickly (several minutes) than the development of tolerance to the antinociceptive effects of opioids (days to weeks), so the relevance to tolerance remains unclear (see above). When both signalling efficacy and this acute desensitization have been directly measured in the same heterologous expression systems (Borgland, 2001), the ability to produce desensitization was directly correlated with receptor-signalling efficacy, albeit rightward shifted. This finding contrasts with the behavioural data described above showing that high-efficacy μ-opioid receptor agonists produce less tolerance than low-efficacy agonists (Madia *et al.*, 2009). In other words, high-efficacy μ-opioid receptor agonists produce the least tolerance, but the greatest desensitization (Table 4). For example, morphine produces rapid tolerance, but relatively little desensitization to inhibition of GIRK currents in locus coeruleus neurons (Dang and Williams, 2005). These data suggest that acute desensitization may not be a good predictor of tolerance to the antinociceptive effects of opioids. Of course, this conclusion may depend on the signalling pathway involved. Several studies indicate that different mechanisms underlie shot-term tolerance to high- and low-efficacy agonists (Hull *et al.*, 2010; Melief *et al.*, 2010). Thus, a causal relationship between acute desensitization and tolerance may yet be established. One possibility is that desensitization contributes to tolerance to high-, but not low-efficacy agonists.

The experimental data are clear about one thing; tolerance to opioids is rapid and pronounced. As described above, this is evident at both the behavioural and cellular level. Although the long-term use of opioids to treat chronic pain suggests that pain may interfere with the development of tolerance to opioids in humans (Cowan *et al.*, 2001; Portenoy *et al.*, 2007), data derived from both animals and drug abusers demonstrate that tolerance is a real potential problem with opioid use. The two most interesting findings are that different signalling mechanisms may contribute to tolerance to different opioids and the magnitude of tolerance is less with high- compared with low-efficacy μ-opioid receptor agonists in animal studies. Both of these findings have important clinical implications and need to be examined in human studies.

Addiction

Drug addiction is defined as an uncontrolled craving for a substance and is manifested in drug-seeking behaviours. Opioid addiction, heroin addiction in particular, has been well characterized scientifically (Rosenberg, 2009) and portrayed to the public in graphic detail in movies such as *Trainspotting* and *Requiem for a Dream*. The power of opioids to motivate behaviour is clearly evident in both cases. The

Table 4

Relative efficacy to induce μ-opioid receptor desensitization

Tissue	Rank order to induce desensitization	Citation
LC slice K^+ currents	ME = etorphine = oxymorphine = morphine > oxycodone	Arttamangkul *et al.* (2008)
LC slice K^+ currents	Etorphine > methadone = ME > dermorphin > morphine	Alvarez *et al.* (2002)
Oocytes K^+ currents	Etorphine > sufentanil > DAMGO > methadone > morphine	Yu *et al.* (1997)
Oocytes K^+ currents	DAMGO = fentanyl > morphine	Kovoor *et al.* (1998)
AtT20 cells Ca^{2+} currents	DAMGO ≥ methadone > morphine > pentazocine	Borgland *et al.* (2003)

DAMGO, D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin; LC, locus coeruleus; ME, met-enkephalin.

one exception is the relatively low incidence of addiction to opioids in chronic pain patients (Fishbain *et al.*, 1992; Noble *et al.*, 2010). The lack of addiction in this case could be caused by pain interfering with the rewarding properties of opioids, limited liability as a result of the type of opioid and route of administration used to treat pain (e.g. fentanyl patch vs. intravenous heroin), or restraint in dosing because of the fear of addiction. Animal studies are well suited to test these hypotheses. Given that addiction is defined by craving, experimental models are limited to behavioural approaches in intact animals. A number of molecular mechanisms that contribute to drug addiction have been described and are reviewed elsewhere (Nestler, 2004). Although these animal models of addiction have limitations, they have good predictive validity for abuse liability in humans.

Self-administration is the best and most commonly used model of opioid addiction in animals. These studies typically require an animal to press a lever to receive a drug. The drug is usually delivered through an intravenous catheter, although other routes of administration such as oral or intracranial can be used. Most self-administration studies use rats, but the procedure is similar whether the experimental subjects are transgenic mice or humans. The most striking finding is that animals will reliably press a lever to self-administer the same drugs abused by humans (Johanson and Balster, 1978; Balster, 1991; Brady, 1991). Although there are a number of variations to this approach such as different reinforcement schedules, the use of second ordering conditioning and direct intracranial drug administration (Richardson and Roberts, 1996; McBride *et al.*, 1999; Schindler *et al.*, 2002), the key feature is that the animal has control of drug intake.

The validity of self-administration studies in animals as a way to assess the reinforcing properties of drugs has been validated in self-administration studies in humans (Haney, 2009). The procedure for self-administration of opioids in humans is similar to that in animals except that human administration can be via intravenous or intranasal routes, human volunteers are typically abusing opioids prior to the start of the study, and subjects are given a choice between repeat administration of an opioid and cash as a way to assess the magnitude of reinforcement (Haney and Spealman, 2008; Comer *et al.*, 2008a). Almost all opioids produce an increase in responding using these procedures, although tolerance appears to limit the reinforcing properties of some opioids, such as buprenorphine, in subjects maintained on opioids (Winger and Woods, 2001; Comer *et al.*, 2008b). The subjective potency ranking for 'good effects' following opioid self-administration puts fentanyl at the top, followed by buprenorphine and heroin, and then morphine and oxycodone (Comer *et al.*, 2008b). The high subjective rating for buprenorphine is probably related to its ability to limit opioid withdrawal because it was not self-administered at levels greater than placebo. Similar potency ratings for opioid self-administration have been reported in rhesus monkeys (Winger and Woods, 2001).

Opioid self-administration has proven useful in testing treatments for opioid addiction. The three currently approved drug treatments for addiction, methadone, buprenorphine and naltrexone, have similar effects in human addicts and heroin self-administration studies (Haney and Spealman, 2008). Thus, self-administration studies in animals are a useful tool to screen potential treatments for opioid addiction.

Conditioned place preference (CPP) also has been used to assess the abuse potential of drugs. The conditioning part involves pairing drug administration with a specific environment and the drug vehicle with a different environment. The rewarding properties of the drug are assessed by allowing the rat to move between the two environments in the absence of drug. An increase in the amount of time spent in the drug-paired environment indicates that the previously administered drug has rewarding properties. A preference has been shown to occur following a single pairing with morphine (Bardo and Neisewander, 1986), although the magnitude of the preference increases with the number of conditioning trials (Lett, 1989).

The primary problem with CPP is that the relationship to drug abuse is not clear. A preference indicates that a drug is rewarding, but it is not known whether environments associated with drug administration in humans are preferred as would be predicted by CPP studies in animals. Nonetheless, most drugs that are self-administered by animals also produce

CPP (Bardo and Bevins, 2000). This correspondence between self-administration and CPP appears to be particularly good for opioids. That is, opioids appear to be rewarding whether self-administered or passively injected during place conditioning. The main advantage of CPP over the self-administration procedure is that preference is assessed in the absence of drug because no drug is administered on the test day. Thus, locomotor and other unconditioned effects produced by opioids do not confound CPP.

Animal models also have been developed to study drug relapse. A major problem with inpatient treatment programmes for drug addiction is relapse when the person returns to the 'drug' environment (Carter and Tiffany, 1999). Both self-administration and CPP procedures have been used to study reinstatement of drug-seeking behaviour (Lu *et al.*, 2003; Crombag *et al.*, 2008). Reinstatement requires an initial training phase in which the drug is paired with lever pressing or a specific environment, followed by extinction trials in which drug is not administered. Reinstatement occurs when the animal is re-exposed to the drug, cues associated with the drug, other drugs or environmental stressors (Shalev *et al.*, 2002). Similar stimuli produce relapse in humans (Carter and Tiffany, 1999; Sinha, 2001). Most of what is known about relapse comes from studies using heroin and cocaine (Shalev *et al.*, 2002; Bossert *et al.*, 2005). The studies described above indicate that animal studies of addiction and relapse are a good model for these behaviours in humans.

Dependence and withdrawal

Although the term dependence may be used more or less interchangeably with addiction, it can be defined as the presence of withdrawal signs upon removal of the drug, as it is here. Abrupt cessation of chronic opioid use or challenge with μ-opioid receptor antagonists during continued treatment produces a highly aversive withdrawal syndrome with features that are similar in humans and a number of experimental animals. In humans, the withdrawal syndrome consists of signs and symptoms including stomach cramps, diarrhoea, rhinorrhoea, sweating, elevated heart rate and increased blood pressure, irritability, dysphoria, hyperalgesia and insomnia (Cushman and Dole, 1973). After abrupt cessation of heroin or morphine use, the withdrawal syndrome develops over a period of less than 1 day and generally persists with declining severity for 1 week to 10 days. However, dysphoria and anhedonia can persist for much longer. The withdrawal syndrome contributes to opioid addiction during cycles of opioid use or abuse presumably because repeated dosing is maintained or escalated to avoid the withdrawal syndrome leading to development of more profound tolerance and dependence (Koob and Le Moal, 1997). Since the identification of cellular adaptive processes that could mediate opioid withdrawal (e.g. discovery of hypertrophy of adenylate cyclase signalling nearly four decades ago), a large range of cellular/biochemical mechanisms that may contribute to the opioid withdrawal syndrome have been identified (Williams *et al.*, 2001; Christie, 2008). However, the extent to which the adaptations that produce withdrawal contribute to opioid addiction is uncertain because relapse is common long after the withdrawal syndrome abates and distinct neural systems appear to be involved in addiction and dependence (Koob and Le Moal, 1997; Christie, 2008).

Animal models of opioid withdrawal have been utilized in the hope of finding opioids that might induce less withdrawal and perhaps have lower addiction liability (Berger and Whistler, 2010). Animal models have very strong predictive validity that the same opioids will produce dependence in humans. As has been proposed for tolerance development, both agonist receptor efficacy and endocytosis efficacy have been implicated in the propensity for dependence to develop (Berger and Whistler, 2010). Of course, pharmacokinetic/pharmacodynamic equivalence would need to be established to make such comparisons (see above) but this has generally not been achieved. More importantly, it is not known if opioid efficacy has any impact on dependence in humans. All potent opioid agonists produce dependence in humans, regardless of their efficacy for receptor signalling or internalization, but whether or not severity of dependence for equieffective opioid doses (and duration of action) of these differs is not known in humans or animals.

Animal models also have been used to examine a range of adjuncts that can lessen the severity of withdrawal with the goal of facilitating opioid detoxification. For example, animal models have led to the use of the α2-adrenoceptor agonists clonidine and lofexidine to reduce the severity of opioid withdrawal in humans (Berger and Whistler, 2010).

The signs of opioid withdrawal in rodents include those referred to as somatic or vegetative signs, as well as aversive signs. The distinction is somewhat artificial although some signs are clearly mediated predominantly by adaptations in peripheral nerves while others are centrally mediated (Koob *et al.*, 1992). In rats and mice, opioid withdrawal signs include jumping, burrowing, 'wet-dog' shakes, hyperreactivity, vocalization, teeth chatter, piloerection, ptosis, lacrimation, rhinorrhoea, diarrhoea, abrupt weight loss, penile erection and ejaculation (Laschka *et al.*, 1976; Koob *et al.*, 1992). These signs are readily quantified following administration of antagonists such as naloxone (termed 'naloxone-precipitated withdrawal') or after abrupt cessation of treatment with relatively short-acting opioids (termed 'spontaneous withdrawal').

A number of factors should be kept in mind when studying withdrawal. Spontaneous or naloxone-precipitated withdrawal can be difficult to observe after chronic administration of very long-acting, high-affinity opioids such as buprenorphine despite development of considerable tolerance because displacement of the receptor bound agonist by naloxone is difficult to achieve (Dum *et al.*, 1981). In addition, the dose of naloxone must be sufficient to adequately compete with the concentration of the opioid agonist present in the vicinity of μ-opioid receptors (1–5 $mg \cdot kg^{-1}$ is usually sufficient) but not so high that off-target effects of naloxone are produced. The testing environment also affects the expression of some withdrawal signs. Jumping (presumably escape attempts) is expressed frequently during withdrawal in both rats and mice in environments such as large vertical observation cylinders but not in small cages. Burrowing (presumably an escape behaviour) is most clearly observed where the opportunity to burrow is provided by the presence of abundant bedding in the observation chamber. Some signs such as ptosis and wet-shakes are more readily scored in rats

than mice. Some authors incorporate the scores for different signs into a 'global' withdrawal scale (Koob *et al.*, 1992), but this can be problematic when investigating the capacity of different opioids or adjuncts to affect withdrawal because some signs may be affected more than others. Experimental models of the aversive nature of opioid withdrawal generally involve tests of conditioned place aversion (Koob and Le Moal, 1997). This approach appears to be more sensitive to low doses of antagonists than expressed signs, but the basis for this difference is not understood.

Although most signs considered characteristic of opioid withdrawal are not expressed in the continued presence of opioid agonists, some signs reflect adaptive mechanisms that persist in the continued presence of opioid agonists but also are exacerbated during abstinence. For example, opioid hyperalgesia can develop after chronic opioid use in both animals and humans and appears to be due to adaptations produced by chronic agonist exposure in the medulla that control descending pain modulatory mechanisms (Fishbain *et al.*, 2009). It is therefore important when examining 'opioid hyperalgesia' as distinct from 'withdrawal hyperalgesia' to establish whether or not any other signs of opioid withdrawal are expressed. This can be difficult when chronic treatment involves use of high doses of very low-efficacy agonists (e.g. buprenorphine) that can themselves precipitate withdrawal (Lobmaier *et al.*, 2010).

Concluding remarks

Although preclinical measures of opioid antinociception, tolerance, addiction and dependence need to be interpreted with caution, overall they appear to have good predictive validity for human responses to opioids. The primary problem in making this connection is the lack of randomized controlled human studies. Even the best human studies do not compare differences in analgesic efficacy or tolerance liability for different opioids. In contrast, animal studies reveal clear differences in antinociceptive efficacy and liability for tolerance depending on the opioid. Moreover, the opioids producing these different effects have been linked to different signalling cascades. The future promise of these cellular studies is the identification of specific signalling pathways for antinociception, tolerance, addiction and dependence. This knowledge will allow new compounds to be screened that stimulate the signalling pathways involved in antinociception, but not those contributing to tolerance, addiction or dependence. What is less certain is how well preclinical findings for opioids that differentially signal to different intracellular cascades, preferentially act on more than one receptor type, or act on heteromultimers will translate to humans. Animal models of tolerance are most useful when combined with parallel analyses of opioid sensitive tissues and neurons from tolerant animals as well as other cellular models that can be probed for receptor function and signalling. Cellular models are limited when used alone because the relationship of specific neurons or signalling mechanisms to analgesia, tolerance, addiction and withdrawal is difficult to know. However, the relative efficacy of various opioids is consistent across many experimental approaches and signalling pathways suggesting that these techniques have good clinical relevance.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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