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REVIEW

Efficacy and ligand bias at the μ-opioid receptor

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In order to describe drug action at a GPCR, a full understanding of the pharmacological terms affinity, efficacy and potency is necessary. This is true whether comparing the ability of different agonists to produce a measurable response in a cell or tissue, or determining the relative ability of an agonist to activate a single receptor subtype and produce multiple responses. There is a great deal of interest in the μ-opioid receptor (MOP receptor) and the ligands that act at this GPCR not only because of the clinically important analgesic effects produced by MOP agonists but also because of their liability to induce adverse effects such as respiratory depression and dependence. Our understanding of the mechanisms underlying these effects, as well as the ability to develop new, more effective MOP receptor drugs, depends upon the accurate determination of the efficacy with which these ligands induce coupling of MOP receptors to downstream signalling events. In this review, which is written with the minimum of mathematical content, the basic meaning of terms including efficacy, intrinsic activity and intrinsic efficacy is discussed, along with their relevance to the field of MOP receptor pharmacology, and in particular in relation to biased agonism at this important GPCR.

LINKED ARTICLES

Recent reviews on aspects of efficacy can be found at:

Kenakin, T (2013). New concepts in pharmacological efficacy at 7TM receptors: IUPHAR Review 2. British Journal of Pharmacology 168: 554–575. doi: 10.1111/j.1476-5381.2012.02223.x

Roche D, Gil D and Giraldo J (2013). Mechanistic analysis of the function of agonists and allosteric modulators: reconciling two-state and operational models. British Journal of Pharmacology 169: 1189–1202. doi: 10.1111/bph.12231

Abbreviations

DAMGO, [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin; β-FNA, β- funaltrexamine; RGS, regulator of G protein signalling

Introduction

When discussing data regarding opioid receptor signalling with other biomedical scientists or clinicians, particularly those with no formal pharmacological training, it is noticeable how often they misunderstand the meaning of terms such as efficacy and receptor reserve. These are important concepts in light of the increasing efforts to identify biased ligands for opioid receptors and other GPCRs because the accurate determination of efficacy is essential for the detection of ligand bias. Taking the clinically important analgesic drug morphine as an example, the fact that this drug can readily induce some cell signalling responses, yet is poor at inducing μ-opioid receptor (MOP receptor) internalization, is taken by many as evidence that morphine is fundamentally different from other MOP receptor agonists (receptor nomenclature follows Alexander *et al.*, 2011). However, as will be discussed later, such apparently striking differences may, in some cases, be explained by more mundane factors, such as differing receptor reserves for distinct responses generated by an agonist at a single receptor subtype. Another issue is that some of the research articles and reviews covering efficacy and receptor reserve can be fairly impenetrable for those who have limited pharmacological or mathematical experience. In this review, and beginning with the simpler concepts of receptor theory, I have attempted to explain what efficacy is, how efficacy at MOP receptors or any other GPCR can be measured and how efficacy can then be used to identify biased ligands. The initial sections of this review cover basic material, and the reader who is familiar with these concepts may like to skip these paragraphs.

The idea of efficacy

The potency of an agonist in producing a response, usually represented by its EC_{50} value (Figure 1A), is a function of both the affinity and the efficacy of the agonist at the receptor. Efficacy itself can be described as the ability of a drug, once bound to a receptor, to activate the receptor and produce a
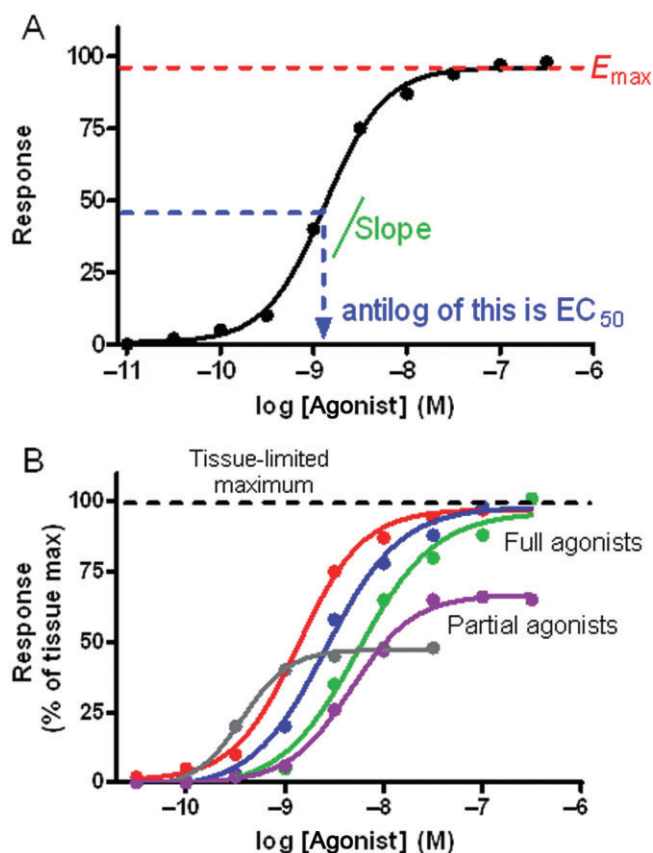
Figure 1

(A) Agonist action is usually shown as a log concentration–response curve, with the curve being defined by the agonist potency (EC_{50} value), the agonist maximum response (E_{max}; this may or may not be the tissue maximum) and also by the slope at the midpoint of the curve. (B) Diagrammatic representation of a family of agonist concentration–response curves produced by activation of a single receptor subtype in a tissue. Note that since partial agonists have low efficacy for the response measured, then the partial agonist represented by the grey curve in (B) must have a very high affinity in order to have such a high potency (i.e. low value of EC_{50}). Buprenorphine activation of [^{35}S]GTPγS binding would be an example of such a MOP receptor agonist (McPherson et al., 2010).

cellular response. However, the measurement of drug efficacy is not always straightforward and, for full agonists, efficacy cannot be read directly from a concentration–response curve. This is because, although the maximum response that an agonist produces is related to its efficacy, the maximum response is also often tissue-limited. A tissue limit could be imposed by the concentration of G protein or effector enzyme present. Also, tissue limits occur where, for example, an agonist induces complete inhibition of a response such as transmitter release or muscle contraction before maximum activation of the receptors has occurred. In addition, with opioids, other factors may influence the maximum response measurable. With analgesia testing in rodents, for example, ethical considerations limit the amount of time a painful stimulus can be applied, with the result that many MOP receptor agonists considered to have relatively low efficacy at

the receptor, appear as full agonists in these assays (Madia et al., 2009; Hull et al., 2010). Consequently, for the various reasons described previously, the maximum response of an agonist in a tissue can, in some cases, be reached by occupying only a fraction of the available receptors present in the tissue. In the case illustrated in Figure 1B, three of the agonists produce the same tissue maximum response, but they could have quite different efficacy values. In the tissue where the experiment was undertaken, these agonists would be referred to as 'full agonists', and the reason why they have different potencies (i.e. different EC_{50} values) is because they have different affinities, different efficacies or both. In other words, because potency depends upon both affinity and efficacy, the agonist depicted in red in Figure 1B is the most potent of the full agonists for the response because its combination of affinity and efficacy is greater than the other two full agonists.

In some cases, agonists have such low efficacy that they cannot achieve the maximum response that a full agonist does, even when occupying all the receptors present in the tissue (Figure 1B); these agonists are called partial agonists. For example, with MOP receptors, ligands such as buprenorphine, meperidine and pentazocine behave as partial agonists in many cell signalling assays (McPherson et al., 2010). The idea that agonists can produce a maximum response in a tissue by occupying only a fraction (less than 100%) of the available receptors leads to the idea of receptor reserve. An agonist with high efficacy will thus have a greater receptor reserve than one with lower efficacy, and an agonist that does not have sufficient efficacy to produce the tissue maximum (i.e. a partial agonist) has no receptor reserve. For example, if one of the full agonists in Figure 1B was able to produce the maximum tissue response by occupying 8% of the available receptors in the tissue, then the receptor reserve would be 92%; if another of the full agonists did so by occupying 95% of the available receptors, then the receptor reserve would only be 5%. Even at the highest concentrations, the partial agonists can only produce a maximum response of less than 100% of the tissue maximum, so have receptor reserves of zero.

One way to investigate the presence of a receptor reserve is to inactivate a fraction of receptors in a tissue with an irreversible receptor antagonist (Nickerson, 1956) to see how this affects the agonist concentration–response curve. This approach has also been used for MOP receptors, with irreversible MOP receptor inactivators such as β-funaltrexamine (β-FNA), β-chlornaltrexamine and clocinnamox (Chavkin and Goldstein, 1982; Williams and North, 1984; Mjanger and Yaksh, 1991; Chan et al., 1995). If irreversible inactivation of a fraction of receptors is performed for two full agonists that have the same efficacy, then the concentration–response curves will be affected in the same way; if they have different efficacies, then the curves will behave differently following receptor inactivation. In the example shown in Figure 2A, agonists R and B have similar potency values and produce the same maximum response, but R has higher efficacy than B. Pretreatment with a low concentration of an irreversible antagonist shifts the curve for agonist R to the right but does not affect the maximum response to the drug, while for agonist B, there is little shift, but the maximum response decreases markedly (Figure 2B,C). With a higher concentration of irreversible antagonist to inactivate more receptors,
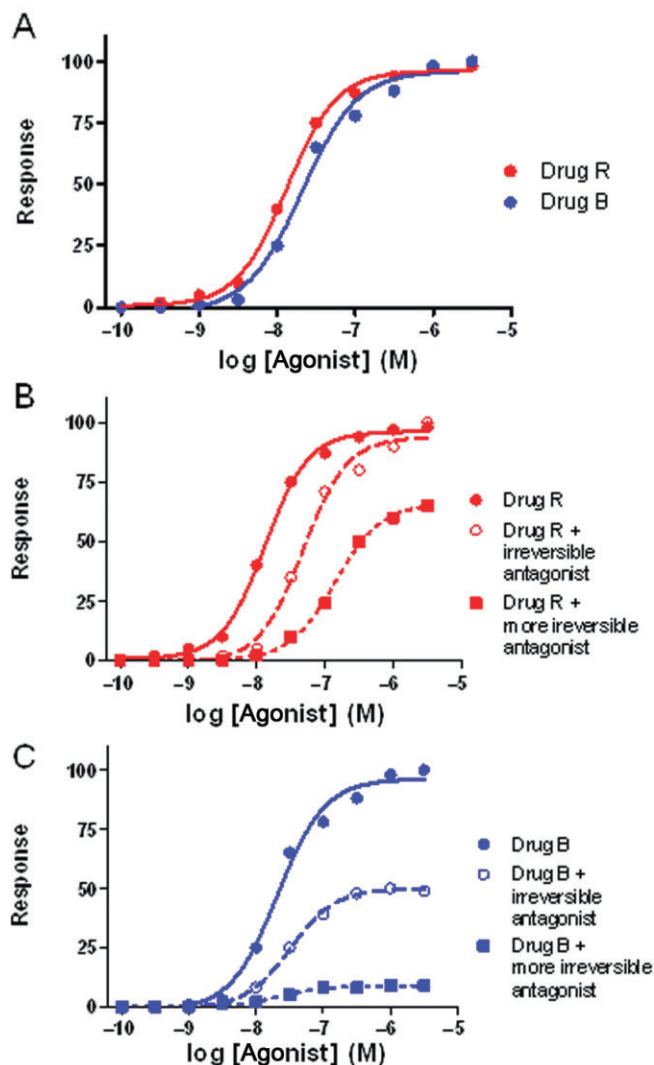
Figure 2

Agonists can have different receptor reserves for a response. (A) Diagrammatic representation of two agonists, R and B, which act at the same receptor subtype and are full agonists for the response measured. (B) Following pretreatment of the tissue with a low concentration of an irreversible antagonist (e.g. β-funaltrexamine for MOP receptors) the curve for agonist R shifts to the right but the maximum response stays the same. Pretreatment with a higher concentration of the irreversible antagonist further shifts the curve to the right but also reduces the maximum response by about 35%. (C) Pretreatments of the tissue with the irreversible antagonist produce little shift of agonist B, but the maximum response is markedly reduced, with almost no response recordable after the higher concentration of irreversible antagonist. The receptor reserve for agonist R must be significantly greater than the one for agonist B, with the receptor reserve for agonist B being very small as there is a little rightward shift of the curve for the agonist before the maximum response decreases.

the maximum for agonist R now decreases, while the curve for agonist B collapses almost completely. The reason why R initially shifts to the right with no drop in maximum is that there are still sufficient receptors left for agonist occupation

to give the maximum response; however, as there are fewer receptors left after inactivation, so a higher concentration of agonist is needed to occupy the required number of receptors. From the concentration–response curves in Figure 2, we can conclude that the concentration of receptor (R_T) in a tissue is a key determinant of the relationship between agonist concentration and response. In addition, there is a larger receptor reserve for agonist R than agonist B, and, consequently, agonist R has a higher efficacy than agonist B, even though in the untreated tissue agonists R and B produce the same E_{max}. Treatment of rats with irreversible inactivators of MOP receptors have shown, for example, that for opioid-induced analgesia, there is a larger receptor reserve for [D-Ala[2], NMe-Phe[4], Gly-ol[5]]-enkephalin (DAMGO) than for morphine (Mjanger and Yaksh, 1991), indicating that DAMGO has higher efficacy than morphine for this MOP receptor-mediated response. The inactivation technique can also be used in other ways. Irreversible inactivators have been used to demonstrate that chronic morphine treatment, presumably via some form of desensitization, reduces the receptor reserve for MOP receptor agonist-induced responses (Chavkin and Goldstein, 1984; Christie et al., 1987).

Further accessible discussions of efficacy, receptor reserve and related issues are available in the literature (Ruffolo, 1982; Clarke and Bond, 1998; Christopoulos and El-Fakahany, 1999; Strange, 2008; Clarke and Berg, 2010; Stallaert et al., 2011).

Intrinsic activity

Common misconceptions among those who attempt to analyse drug action are that efficacy can always be directly measured from the maximum response to the drug (although note that this is possible in some cases), and that different drugs producing the same maximum response in a tissue have the same value of efficacy. These misunderstandings are surprisingly common and can lead to incorrect conclusions when investigating agonist action. In fact, the maximum response that an agonist produces in a tissue relative to a full agonist is correctly referred to as the *intrinsic activity* of the drug. If two drugs are full agonists in a tissue, then they are said to each have an intrinsic activity of 1. If a drug is a partial agonist (e.g. the purple agonist curve in Figure 1B) and produces a maximum response that is 65% of that of a full agonist, it is said to have an intrinsic activity of 0.65. In general, intrinsic activity does give an indication of efficacy when comparing partial agonists, but it is of little use in the analysis of full agonists because all such agonists will have an intrinsic activity of 1, even though they may well have very different values of efficacy.

Intrinsic efficacy

Efficacy itself is composed of drug-dependent and tissue-dependent components. The drug-dependent component is referred to as the *intrinsic efficacy*, which is the ability of the agonist drug molecule, once bound, to activate the receptor protein, or, put another way, the tendency of the drug to

increase the proportion of the receptor population in an active conformation. The tissue-dependent components of efficacy include the total receptor concentration and the efficiency of coupling the receptor activation to the measured tissue response. These terms are described in a classic equation based mainly on the work of Stephenson (1956) and Furchgott and Bursztyn (1967):

$$\frac{E}{E_{max}} = f\left(\frac{\varepsilon \cdot R_T \cdot [A]}{[A] + K_A}\right) \qquad (1)$$

where E is the agonist response, E_{max} is the maximum tissue response to the agonist, f is some function of the signal produced by the binding of drug to receptor which represents the efficiency of coupling receptor to response, ε is the intrinsic efficacy, R_T is the receptor concentration, $[A]$ is the agonist concentration and K_A is the equilibrium dissociation constant of the drug–receptor interaction. In the equation, ε and K_A are drug-dependent factors, while R_T and f are tissue-dependent factors.

In theory, the relative intrinsic efficacy of one agonist to another for a particular signalling response should be the same irrespective of the tissue where the receptor is expressed. However, even though the intrinsic efficacy may be unchanged, the overall efficacy and hence potency and maximum response (also intrinsic activity) of a drug can vary from tissue to tissue because the factors f and/or R_T, as described in Equation (1), can vary from tissue to tissue (Figure 3). For an example of this, see figure 1 of Selley et al. (1997), where MOP receptor agonist responses are compared in rat thalamus and CHO cells expressing MOP receptors.

In many cases, these considerations do not receive sufficient attention when considering agonist action and often it is assumed, for example, that a full agonist in one tissue will be a full agonist in another, or a partial agonist in one tissue will behave in a similar fashion in all tissues and for all responses examined. In fact, a drug that is a full agonist in one tissue may, due to lower R_T or efficiency of coupling receptor activation to response, have lower potency in

another tissue, or may be a partial agonist or even an antagonist in another (Figure 3). For example, morphine is a full agonist for inhibition of adenylyl cyclase activity in HEK293 cells (Zaki et al., 2000), yet is an extremely weak partial agonist/competitive antagonist in the rat vas deferens preparation (Ishii et al., 1981; Smith and Rance, 1983). In some ways, the situation is not helped by pharmacological databases such as the IUPHAR database (http://www.iuphar-db.org/index.jsp), where morphine is defined as a full agonist at MOP receptors, but whether or not it behaves as a full or partial agonist depends totally upon the assay/tissue being used. This means that it is very important to describe efficacy in terms of both the tissue and the response measured (see Table 1 as an example).

How is drug efficacy quantified?

There are a number of approaches used to determine efficacy (Furchgott and Bursztyn, 1967; Black and Leff, 1983; Ehlert, 1985, 2008; Figueroa et al., 2009; Kenakin et al., 2012; Kenakin and Christopoulos, 2013), or more specifically, the relative intrinsic efficacy of a drug in a tissue (i.e. intrinsic efficacy is usually measured relative to other agonists acting at the same receptor in the same tissue rather than as an absolute quantity for each agonist). Furchgott and Bursztyn (1967) developed a receptor inactivation method that compared agonist concentrations that evoked the same level of response before or after inactivation of a fraction of the total available receptors with an irreversible antagonist. From this, the agonist affinity could be calculated, which was then used to construct log occupancy–response curves, with the relative shift between the curves being taken as a measure of relative intrinsic efficacy; the greater the shift, the greater the difference in agonist intrinsic efficacy. Because the measurements were being made at the same population of receptors in a tissue and because differences in affinity have been accounted for by calculating fractional receptor occupancy, then all tissue-dependent factors (i.e. R_T and f) cancel out, and therefore, differences between the agonists must be due to differences in the intrinsic efficacy, ε.

More simply, it has been proposed by Ehlert (1985) and Strange (2008) that the efficacy of one agonist relative to another can be determined from a simple combination of the difference between K_A and EC_{50}, and the maximum response. As described by Ehlert (1985):

$$e = \left(\frac{E_{max\,agonist}}{E_{max\,full\,agonist}}\right) \times \left\{\left(\frac{K_{A\,agonist}}{EC_{50\,agonist}}\right) + 1\right\} \times 0.5 \qquad (2)$$

where e is the efficacy of the test agonist, and $E_{max\,agonist}/E_{max\,full\,agonist}$ are the relative maximum response values of the test agonist and an agonist giving a full response, while K_A and EC_{50} are the equilibrium dissociation constant and EC_{50}, respectively, of the test agonist.

For the analysis in Equation (2), if efficacy measurements are made for a series of agonists producing a response by acting at the same receptor population in a tissue, then the relative efficacy values (e) calculated become relative intrinsic efficacy values (ε). This approach, which has been used to estimate the relative efficacy of agonists at MOP receptors
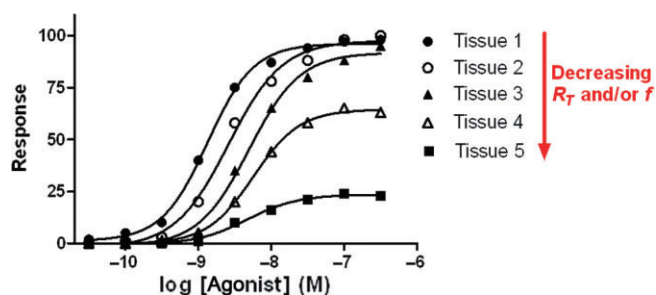

Figure 3

Efficacy is tissue-dependent. Diagrammatic representation of a family of concentration–response curves obtained from different tissues for a response produced by an agonist acting at one particular receptor subtype. Although the agonist has the same value of intrinsic efficacy for the receptor response, variations in the tissue-dependent factors of receptor concentration (R_T) and/or efficiency of coupling of receptor to response can markedly affect the potency and maximum response of the agonist. Note also that decreasing τ from tissue 1→5 would also produce the same family of curves.

Table 1

Intrinsic efficacy of morphine relative to DAMGO (the latter taken as 1 in each case) at MOP receptors for assays involving G protein-mediated responses

Study	Tissue	Assay	Analysis	Efficacy of morphine relative to DAMGO
Emmerson et al. (1996)	C6 glioma cells, rat receptor (heterologous)	[35S]GTPγS binding	Ehlert[a]	0.27
Selley et al. (1998)	CHO cells, mouse receptor (heterologous)	[35S]GTPγS binding	Ehlert[a]	0.72
	Rat thalamus (endogenous)	[35S]GTPγS binding		0.56
Borgland et al. (2003)	AtT20 cells, mouse receptor (heterologous)	Ca²⁺ channel inhibition	Null[b,c]	0.58
			Midpoint shift[c]	0.74
Rónai et al. (2006)	Mouse vas deferens (endogenous)	Inhibition of field-stimulated contraction	Null[b]	0.28
			Operational	0.07
McPherson et al. (2010)	HEK293 cells, rat receptor (heterologous)	[35S]GTPγS binding	Operational	0.18
Nickolls et al. (2011)	U2OS osteosarcoma cells, human receptor (heterologous)	[35S]GTPγS binding	Operational	0.57
		cAMP inhibition		0.27
Rivero et al. (2012)	Rat locus coeruleus neurones (endogenous)	K⁺ current activation	Operational	0.02
Madia et al. (2012)	Rat spinal cord (endogenous)	[35S]GTPγS binding	Operational	0.07

[a]As analysed by Equation (2) above.
[b]Based on the method of Furchgott and Bursztyn (1967).
[c]As described in Borgland et al. (2003).
Taking all the values in the table, the intrinsic efficacy of morphine was 0.36 ± 0.07 (mean $\pm$ SEM) relative to DAMGO; if only the results from operational analysis are included, this becomes 0.19 ± 0.08, and if only the results from the non-operational analysis are included this becomes 0.52 ± 0.08.

(Emmerson et al., 1996; Selley et al., 1998; Rivero et al., 2012), is more staightforward than Furchgott's as the widespread availablility of radioligands makes the measurement of K_A, which is necessary for the calculation in Equation (2), a simple procedure. However, as the K_A value obtained from a membrane preparation can vary depending upon the presence or absence of, for example, guanine nucleotide or Na⁺ ions, then whether a particular K_A value obtained under such conditions is the appropriate one to use to calculate efficacy is another matter. This issue is discussed further below.

An important method developed by Black and Leff (1983) to analyse agonist action is the operational model of agonism, where data from agonist concentration–response curves are fitted to the following equation:

$$\frac{E}{E_{max}} = \frac{\tau^n \cdot [A]^n}{(K_A + [A])^n + \tau^n \cdot [A]^n} \qquad (3)$$

where E is the agonist response, E_{max} is the maximum possible tissue response to the agonist (which can be greater than the measured E_{max}), n is the slope factor of the transducer function, $[A]$ is the agonist concentration, K_A is the equilibrium dissociation constant of the drug–receptor interaction and τ is the operational efficacy, which is R_T/K_e, where R_T is the receptor concentration and K_e is the concentration of the agonist–receptor complex that produces a half-maximal response (note K_e is not the same thing as the EC$_{50}$).

The τ value, or transducer function, is also referred to as the operational efficacy of the agonist and is the ratio of R_T/K_e. Although τ has both tissue-dependent and tissue-independent components, if relative τ values are calculated for agonist responses at a receptor population in a cell type or tissue, then the tissue-dependent components cancel out and relative τ becomes a measure of relative intrinsic efficacy. The τ values for agonists can be calculated by simultaneously fitting all agonist concentration–effect curves to the equation, using K_A values obtained from separate binding assays. Alternatively, the τ value of an agonist can be obtained by fitting the curve for agonist before and after inactivation of a fraction of receptors. In this case, it is not necessary to first determine K_A as the fitting will give a value of this parameter; this may be an advantage if there is concern about using K_A values determined from separate binding assays. In summary, the operational model represents an important and increasingly popular approach to quantify agonist action, and indeed Black and Leff's approach has already been used to analyse MOP receptor agonist efficacy and MOP receptor desensitization in a number of experimental settings (Osborne and Williams, 1995; Cox et al., 1998; Garrido et al., 2000; Rónai et al., 2006; Bailey et al., 2009; McPherson et al., 2010; Nickolls et al., 2011; Madia et al., 2012; Rivero et al., 2012).

Ligand bias

There is enormous interest in the phenomenon of biased agonism (also known as functional selectivity, protean

agonism and ligand-directed signalling; Urban *et al.*, 2007), not least because it offers ways in which more effective drug treatments can be developed. For example, a biased agonist at a receptor might activate a signalling pathway that leads to the desired clinical response but would not activate a signalling pathway responsible for adverse effects (Rajagopal *et al.*, 2010; Whalen *et al.*, 2011), whereas an unbiased agonist would activate both pathways and produce both desired and adverse effects. In many cases, bias is described in terms of signalling via G protein versus arrestin, although bias, in fact, refers to any signalling pathway including those involving different subtypes of G protein or arrestin. Currently, in the field of GPCR research, there is accumulating evidence that bias is widespread (Kahsai *et al.*, 2011; but see Langemeijer *et al.*, 2013). Whatever the case, the measurement of agonist efficacy is of central importance when trying to determine possible ligand bias for signalling pathways. This is, in part, because bias is usually not absolute and it is probably rare for a biased ligand at a GPCR to fully activate one pathway and be completely inactive at another. Accordingly, bias may not be obvious from cursory inspection of concentration–response curves, while differences in receptor reserve for distinct responses emanating from the same receptor can produce data that resemble bias, but closer analysis reveals otherwise. Therefore, rigorous methods to determine efficacy for different signalling pathways are needed, as well as methods to quantify any bias that may be present. Such approaches have now been developed (Rajagopal *et al.*, 2011; Kenakin *et al.*, 2012) and implemented (Evans *et al.*, 2011; Nijmeijer *et al.*, 2012; Rivero *et al.*, 2012; Wacker *et al.*, 2013). It will be of interest to see whether these different approaches lead to similar conclusions with regard to the bias displayed by particular agonists at a GPCR.

An important recent development is the proposal that analysis of concentration–response curves alone can provide adequate quantification of agonist signalling in order to determine ligand bias. These measures, which include components of agonist affinity and efficacy, include the 'intrinsic relative activity' (RA$_i$; not to be confused with intrinsic activity as described earlier) as developed by Ehlert (Griffin *et al.*, 2007; Ehlert, 2008; Figueroa *et al.*, 2009), and the 'transduction coefficient' or 'transduction ratio', which is the ratio τ/K_A as developed by Kenakin and Christopoulos (Kenakin *et al.*, 2012; Kenakin and Christopoulos, 2013). These quantities can be obtained by direct fitting of agonist concentration–response curves to the relevant equations. A significant advantage of these approaches is that independent measures of ligand affinity or partial inactivation of the receptor population is not required to obtain the measures of agonist signalling (Griffin *et al.*, 2007; Ehlert, 2008; Figueroa *et al.*, 2009; Stallaert *et al.*, 2011; Kenakin *et al.*, 2012; Kenakin and Christopoulos, 2013).

These approaches are constantly evolving (Ehlert *et al.*, 2011; Kenakin, 2013; Kenakin and Christopoulos, 2013), and although the theoretical basis can seem complex, such ideas are likely to see increased application in the future because of their importance in assessing ligand bias. Whichever approach is used to determine ligand bias at a receptor, with modern computer packages now available, and with practice and advice (e.g. Hall and Langmead, 2010), it should be possible for the experimenter to fit data to these models.

Context-dependent efficacy

Another important consideration is that of context-dependent efficacy. This relates in many ways to the efficiency of coupling. Here, the efficacy is dependent upon the presence of other factors that may be present or absent in a cell, or present at very different concentrations, such as particular proteins that interact with the receptor. A clear example of this is receptor phosphorylation. Thus, the efficacy of arrestin recruitment is, in most cases, increased by receptor phosphorylation by G protein coupled receptor kinases (GRKs). Accordingly, when arrestin-dependent signalling is being examined, the ligand efficacy will be greatly influenced by the concentration of the relevant GRK and phosphatase subtypes present in the cell type, or even subcellular compartment of the cell. A good example of this is morphine's ability to induce MOP receptor trafficking. In the vast majority of cell types, morphine produces little or no MOP receptor internalization, unless GRK2 is overexpressed (Zhang *et al.*, 1998; Schulz *et al.*, 2004). However, in certain cell types, such as striatal neurones (Haberstock-Debic *et al.*, 2005), or in certain neuronal compartments, such as the dendrites of nucleus accumbens neurones (Haberstock-Debic *et al.*, 2003), morphine is able to induce significant trafficking without the need for overexpression of GRKs. Other ways in which cellular context could affect overall efficacy and possibly even the relative intrinsic efficacy of agonists are discussed below (see also Figure 9).

Agonist efficacy for G protein signalling at MOP receptors

The complexities of opioid action in the intact organism mean that it is very important to establish opioid drug efficacy at the receptor (Kelly *et al.*, 2008; Morgan and Christie, 2011), not least because the relative intrinsic efficacy of MOP receptor agonists obtained for complex responses, such as analgesia, is a product of pharmacokinetics, the selectivity of ligands for opioid receptor subtypes, as well as agonist action at MOP receptors and the immediate post-receptor environment. Relative intrinsic efficacy values are also extremely useful when studying MOP receptor desensitization and its relationship to tolerance as changes in intrinsic efficacy as seen in desensitization are a more convenient way to quantify desensitization (Navratilova *et al.*, 2007; Bailey *et al.*, 2009) than having to deal with often complex changes in agonist maximum response and potency. Last but not least, reliable measures of intrinsic efficacy are needed to detect and quantify ligand bias, as described below.

Relative intrinsic activity

In the majority of cases, estimations of the efficacy of MOP receptor ligands have been obtained from measures of relative intrinsic activity (Whistler *et al.*, 1999; Molinari *et al.*, 2010). In some cases, this provides a reasonable estimation of efficacy, for example, in [^{35}S]GTPγS binding assays using brain membranes, where the receptor reserve tends to be small and a good number of ligands behave as partial agonists (Selley

et al., 1998). On the other hand, when using [^{35}S]GTPγS assays with clonal cell lines stably expressing reasonably high levels of receptor, many ligands with lower efficacy behave as full agonists, and thus, intrinsic activity is not a good measure of intrinsic efficacy. Also, for assays where there is a high level of receptor reserve associated with the response, such as MOP receptor-induced inhibition of AC activity, intrinsic activity as a means to estimate ligand efficacy is unable to distinguish high efficacy agonists from agonists that are weak partial agonists in other assay systems. Nevertheless, the use of intrinsic activity to compare MOP receptor agonists remains in widespread use (Molinari *et al.*, 2010; Frölich *et al.*, 2011).

Relative intrinsic efficacy

To determine relative intrinsic efficacy at MOP receptors, we assessed agonist-induced stimulation of [^{35}S]GTPγS binding to membranes of HEK293 cells stably expressing MOP receptors at a moderately high density of 778 fmol·mg^{-1} protein (McPherson *et al.*, 2010). Full concentration–effect curves were constructed for 22 ligands. In addition, to use operational analysis without receptor inactivation, the binding constant (equilibrium dissociation constants, K_A) for each agonist was determined. Using these data, the concentration–response curves were simultaneously fitted in GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) to the operational model, as in Equation (3) above. By sharing the values of E_{max} and n for all agonists, and inserting K_A values for each agonist (this was achieved by using the respective K_A value as the column heading for each agonist in the Graph-Pad Prism data table), fitted values of τ were obtained. DAMGO had the highest τ in this assay (28.5), with Met-enkephalin and methadone also having high values (22.0 and 18.2, respectively); morphine had a much lower τ value (5.2), while opioids such as buprenorphine and pentazocine had extremely low τ values (0.6 and 0.3 respectively). To determine whether the τ values of operational efficacy we obtained in HEK293 cells might reflect those for a G protein-mediated response for endogenous MOP receptors expressed in neurones, we also determined τ values for agonists at MOP receptors expressed in locus coeruleus neurones contained in slices of rat midbrain. The response involved MOP receptor-induced activation of an inwardly rectifying K$^+$ current recorded in whole cell patch-clamp mode (Rivero *et al.*, 2012). For this analysis, we constructed concentration–response curves to agonists in the absence or presence of the irreversible MOP receptor inactivator β-FNA. The pairs of curves for each agonist were again analysed by operational modelling, but this time with K_A, E_m and n values constrained to be shared for each agonist curve before and after receptor inactivation. The relative τ values obtained correlated well with those from HEK293 cells (Figure 4), indicating that relative (not absolute) τ values for agonists at a receptor appear to be tissue-independent, and that in this case at least the relative τ values obtained in a clonal cell line represent those that might be obtained for endogenous receptors in neurones.

It is interesting to compare the relative intrinsic efficacy values obtained in these experiments to those obtained in other studies of MOP receptor-mediated G protein-dependent responses (Table 1). The agonists DAMGO and morphine have been used in all the cited studies, and it can be seen that the intrinsic efficacy of morphine relative to DAMGO is
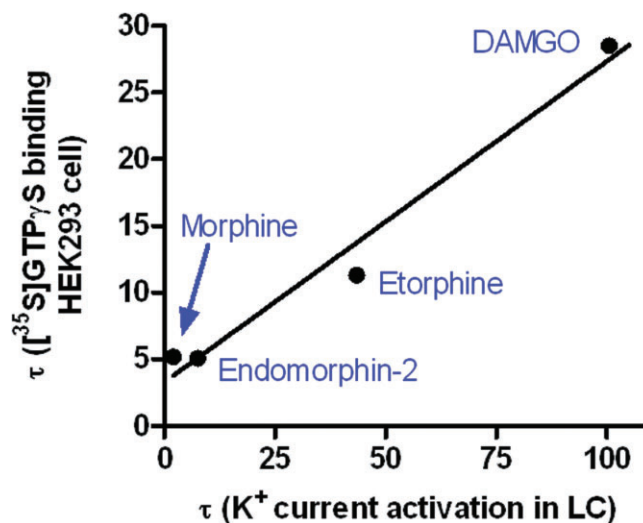

Figure 4

Relative intrinsic efficacy (τ) values for agonists at MOP receptors are tissue-independent. Correlation of τ values for [^{35}S]GTPγS binding in HEK293 cells and activation of a K$^+$ current in locus coeruleus neurones for four MOP receptor agonists. Following linear regression, the r^2 value was 0.975, indicating a high correlation of agonist relative τ values obtained in the two systems. The analysis is based on data from McPherson *et al.* (2010) and Rivero *et al.* (2012).

always significantly less than 1, although there is some variation depending upon the method used. In general, operational analysis results in lower values of intrinsic efficacy for morphine relative to DAMGO, but the reason why morphine's intrinsic efficacy relative to DAMGO might be either underestimated in operational analysis and/or overestimated in other types of analysis is unclear.

As mentioned previously, an important further issue relates to the K_A values used in the operational analysis. In our study, we determined K_A values in the presence of a high concentration of Na$^+$ (137 mM), which is known to stabilize what is likely to be a basal state of the receptor (Strange, 2008). Whether these K_A values are the appropriate ones to use in the operational analysis is an assumption that remains open to question. We used the K_A values determined as above on the basis that since the K_A values of all 22 ligands in our study were obtained by the same method, then on balance this would not significantly affect the overall relative operational efficacy measurements. However, in a recent review, Kenakin and Christopoulos (2013) present evidence that it may not be possible to use a single K_A value obtained from a binding assay to fit data for a single agonist from different functional assays to the operational model. Furthermore, when we used the operational model itself to determine K_A values from concentration–response curves before and after receptor inactivation (Rivero *et al.*, 2012), we found the K_A values obtained to be quite different from those obtained in binding assays. As the K_A values obtained from the operational fitting can be regarded as macroscopic binding constants and those from ligand binding assays as microscopic constants, then it is perhaps not surprising that they are different and therefore should not be directly compared.

These concerns about K_A and its use in this type of analysis remain unresolved (Colquhoun, 1998) and are the subject of recent and continuing discussion (Kenakin, 2013; Kenakin and Christopoulos, 2013).

Agonist efficacy for other signalling outputs from MOP receptors

Apart from G protein-mediated responses, we also wanted to determine the relative intrinsic efficacy of MOP receptor ligands for other signalling events, including arrestin-mediated responses. Apart from mediating the uncoupling of GPCR and G protein, the non-visual arrestins (arrestin-2 and -3) are known to interact with and regulate the function of a large number of signalling proteins such as elements of the MAPK pathway (Luttrell and Gesty-Palmer, 2010; see also Figure 5A). Until relatively recently, quantifying arrestin function has been problematic because of the absence of convenient assays to determine agonist concentration–response relationships for arrestin interaction with a GPCR. However, commercially available arrestin recruitment assays are now available (Bassoni *et al.*, 2012). In addition, the development of FRET (Frölich *et al.*, 2011), BRET (Molinari *et al.*, 2010) and bimolecular fluorescence complementation (Kilpatrick and Holliday, 2012) techniques has enabled the rapid collection of GPCR-arrestin recruitment data. Our own approach (McPherson *et al.*, 2010) was to use the Pathunter system from DiscoveRx (DiscoveRx Corporation, Birmingham, UK), which is a complementation assay where different parts of the enzyme β-galactosidase are fused to either the C-terminal tail of the MOP receptor or to arrestin-3. With this assay, we produced concentration–response curves for all the agonists used in the [35S]GTPγS binding assay described above, and the curves were again subjected to operational analysis and τ values calculated. The absolute τ values were much lower than those for [35S]GTPγS binding but this is not surprising since the potency and/or maximum responses were in all cases lower for the arrestin assay than for G protein coupling. This probably reflects the lack of amplification and hence receptor reserve in the arrestin assay compared to the [35S]GTPγS binding assay. In addition, we were able to use operational analysis to determine the relative intrinsic efficacy for MOP receptor phosphorylation on Ser[375] in the C-terminal tail of the receptor. Phosphorylation of this residue, presumably by a G protein coupled receptor kinase, is a key step in MOP receptor desensitization and trafficking (Schulz *et al.*, 2004; Doll *et al.*, 2012), and a commercially available anti-phosphoreceptor antibody for phosphorylated Ser[375] has been developed. We constructed full agonist concentration–phosphorylation curves for four agonists, and the pattern of relative τ values we obtained for Ser[375] phosphorylation (DAMGO ≥ etorphine ≥ endomorphin-2 > morphine) was the same as that for arrestin-3 recruitment. Absolute τ values were again low and similar to those obtained for arrestin recruitment. Thus, we were able to use operational analysis to determine relative intrinsic efficacy for multiple signalling outputs from a single receptor subtype. The further manipulation and interpretation of these data in relation to biased agonism is discussed below.
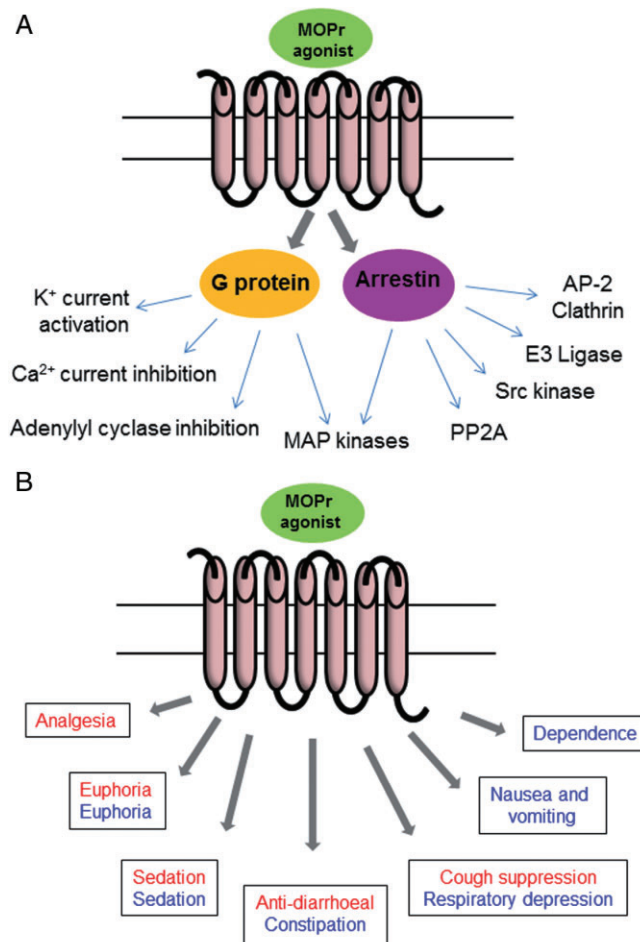

Figure 5

(A) Major cellular signalling pathways activated by MOP receptor (MOPr) agonists. While the G protein-dependent signalling pathways have been established over many years, the arrestin-dependent signalling pathways have only recently begun to be characterized (Walwyn *et al.*, 2007; Groer *et al.*, 2011; Raehal *et al.*, 2011; Henry *et al.*, 2012). The MAPK cascade can be activated via G protein- or arrestin-dependent pathways (Zheng *et al.*, 2008). Many of these pathways also lead to changes in gene expression, particularly with prolonged agonist treatments. (B) The *in vivo* consequences of MOP receptor agonist administration to a patient. Effects in red are desirable, therapeutic effects; those in blue are adverse, undesirable effects. Some, such as sedation or euphoria, can be therapeutic or undesirable depending upon the context.

User-friendliness and suitability of the operational model

How easy is it to fit data to the operational model and obtain reliable values of τ? Operational modelling requires good concentration–response data, particularly if the analysis involves only a small number of ligands; tight data and well-defined maxima all help. By using constraints, such as of the n value or E_{max} value, it can be possible to fit data that at first attempt appears difficult to fit. Often it is helpful to first fit the data to a logistic model (sigmoidal concentration–

response) to obtain estimates of maximum response and n that can be used as initial estimates in the subsequent operational analysis (note, however, that E_m and n do not have exactly the same meaning in the logistic and operational models, but the values obtained from fitting to the logistic model are fine to use as starting points for operational fitting). Both logistic and operational fitting can be undertaken in a suitable data analysis programme such as Graph-Pad Prism, which already includes the equation for the operational model. However, even if the fitting of the data is able to produce τ values, care must be taken to check that the errors associated with fitting are not so large as to make the τ values essentially meaningless. In this regard, our attempts to analyse MOP receptor internalization data using operational modelling were unsuccessful and so we used the approach described by Ehlert, (1985) to estimate relative intrinsic efficacy for this readout (Equation 2 above).

An important question is whether the operational model is actually suitable to analyse concentration–response data for responses (such as arrestin recruitment and receptor phosphorylation) that have little or no receptor reserve. It has previously been suggested that data showing a linear relationship between occupancy and response should not be fitted to the operational model (Nickolls *et al.*, 2011) as the latter relies upon a model where the occupancy–response relationship is hyperbolic. To determine the relationship between occupancy and response, a straightforward approach is to calculate fractional receptor occupancy at each concentration of ligand using the simple relationship:

$$\rho = \frac{[A]}{K_A + [A]} \qquad (4)$$

where ρ is the fractional receptor occupancy, $[A]$ is the concentration of agonist and K_A is the equilibrium dissociation constant. Fitting of the resulting data, for example, to a linear regression or hyperbolic function reveals the nature of the occupancy–response relationship.

For the MOP receptor data described above from Rivero *et al.* (2012), we found that the relationships between agonist fractional receptor occupancy and [35S]GTPγS binding are best described by hyperbolic curves (Figure 6A; see also Figure 7 and Supplementary Figures S3 and S4 from Rivero *et al.* 2012), indicating amplification and a receptor reserve. On the other hand, the relationships between receptor occupancy and either arrestin recruitment, Ser[375] phosphorylation or internalization did not fit well or could not be fitted to a hyperbolic relationship. Rather, the data fitted better to a linear relationship than a hyperbolic one (Figure 6). This is perhaps not surprising because there is unlikely to be much amplification in these signalling readouts, and these responses normally require high levels of receptor occupancy (Borgland *et al.*, 2003). However, it should be noted that the relationship between receptor occupancy and the regulatory readouts may be more complex yet, as, for example, these data could also be readily fitted to an exponential growth model (Figure 6E,F). It is not immediately obvious what this might mean, but possibilities include that there is a high occupancy threshold for these regulatory responses, or that hierarchical phosphorylation (Lau *et al.*, 2011; Just *et al.*, 2013) of the C-terminus of MOP receptors results in a complex occupancy–response relationship, which is accordingly reflected in the processes of arrestin recruitment and receptor internalization.

It may well be that for the three regulatory readouts of phosphorylation, arrestin recruitment and internalization, the E_{max} value of each agonist (i.e. the intrinsic activity) is sufficient as a measure of intrinsic efficacy, as discussed in the section below (also Figure 7A,B). In summary, for responses with no receptor reserve, although operational analysis evidently does provide reasonable estimates of relative intrinsic efficacy (McPherson *et al.*, 2010), it may be an overcomplicated way to treat the data, especially as relative intrinsic activity (as given by relative E_{max} values) is easier to measure.

Biased agonism at MOP receptors

An important reason to obtain accurate measures of agonist relative intrinsic efficacy is to facilitate detection of ligand bias. A large number of ligands exist for MOP receptors and many of the responses to MOP receptor activation, such as ion channel modulation and adenylyl cyclase inhibition are known to be mediated by G proteins of the $G_{i/o}$ subfamily (Figure 5A). On the other hand, the role of arrestin-dependent signalling through MOP receptors is largely unknown, although responses to MOP receptor agonists are significantly modified in arrestin-3 knockout mice (Bohn *et al.*, 1999; Raehal *et al.*, 2005, 2011). Given the importance of this receptor in pain and reward pathways, it is important to evaluate possible ligand bias as a means to explore the role of different signalling pathways in opioid drug action *in vivo* (Figure 5B). For example, could a G protein-biased or arrestin-biased opioid agonist be an effective analgesic but display fewer adverse effects such as dependence? To determine bias, we initially constructed a correlation plot of τ values for arrestin-3 recruitment versus τ values of [35S]GTPγS binding (Figure 7A). When the experimental data were subjected to linear regression, the overall correlation was quite high ($r^2 = 0.646$ for the entire series of MOP receptor ligands; McPherson *et al.*, 2010), indicating that in general, the better the agonist is in promoting coupling to G protein, the better the agonist is at recruiting arrestin. This was not unexpected and has been reported for other GPCRs such as the β_2-adrenoceptor (January *et al.*, 1997). Interestingly, and of relevance to the preceding section, if intrinsic activity values are used instead of τ values for arrestin recruitment, the same relationship is obtained (Figure 7B). However, the same does not hold if intrinsic activity values are used instead of τ values for [35S]GTPγS binding (Figure 7C) because most of the agonists examined were full agonists in this assay and hence had an intrinsic activity of close to 1. Further inspection of the correlation in Figure 7A indicated two points of particular interest. Firstly, it appears that the endomorphins are biased towards arrestin recruitment, and, secondly, that morphine is not biased between G protein and arrestin pathways.

The endomorphins are arrestin-biased

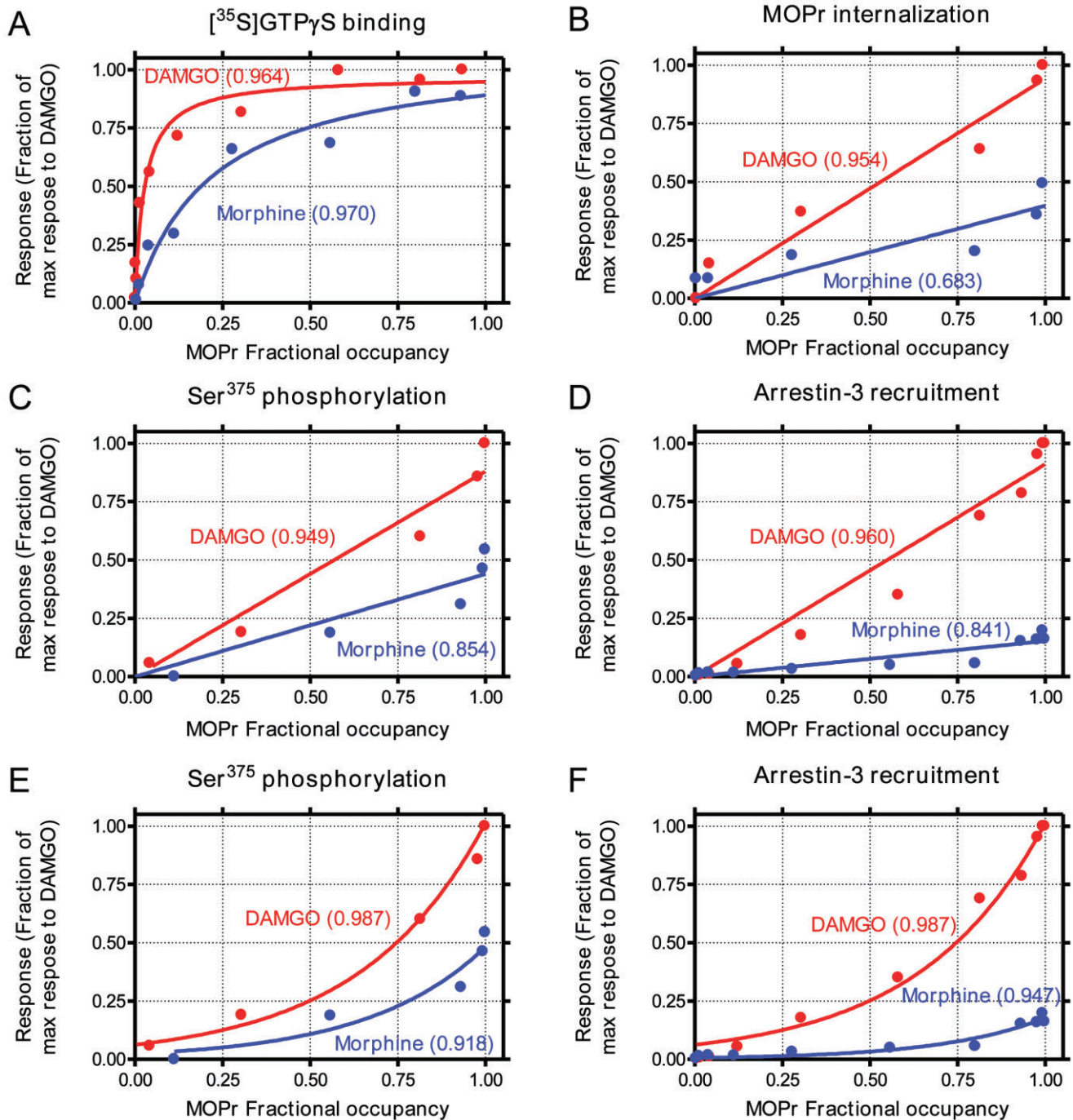That the endomorphins are arrestin-biased was an unexpected finding as the tetrapeptides endomorphin-1 and -2

Figure 6

Occupancy–response relationships for DAMGO and morphine for (A) [^{35}S]GTPγS binding fitted to a hyperbolic function, for (B–D) MOP receptor internalization, Ser^{375} phosphorylation and arrestin-3 recruitment fitted by linear regression, and for (E-F) the Ser^{375} and arrestin-3 data points from C and D instead fitted to an exponential growth model. The values of occupancy were calculated using Equation (4), using data previously published (McPherson *et al.*, 2010; Rivero *et al.*, 2012). The values shown in brackets are the R^2 values from the fits in GraphPad Prism. For linear regression, in all cases the line was constrained to go through $X = 0$, $Y = 0$. The occupancy–response relationships for Ser^{375} phosphorylation, arrestin-3 recruitment and internalization were better described by linear than hyperbolic relationships, indicating little or no receptor reserve for these responses in this assay system (R^2 values for fitting of occupany–response data for Ser^{375} to a hyperbolic function was 0.750 for DAMGO and did not converge for morphine; for arrestin-3 recruitment 0.788 for DAMGO and 0.704 for morphine; for internalization 0.874 for DAMGO and 0.658 for morphine). However, the R^2 values for fitting Ser^{375} phosphorylation (E), arrestin-3 recruitment (F) and internalization (not shown) to an exponential growth model [$Y = Y_0 \times \exp(k \times X)$] was, in turn, better than to a linear model.
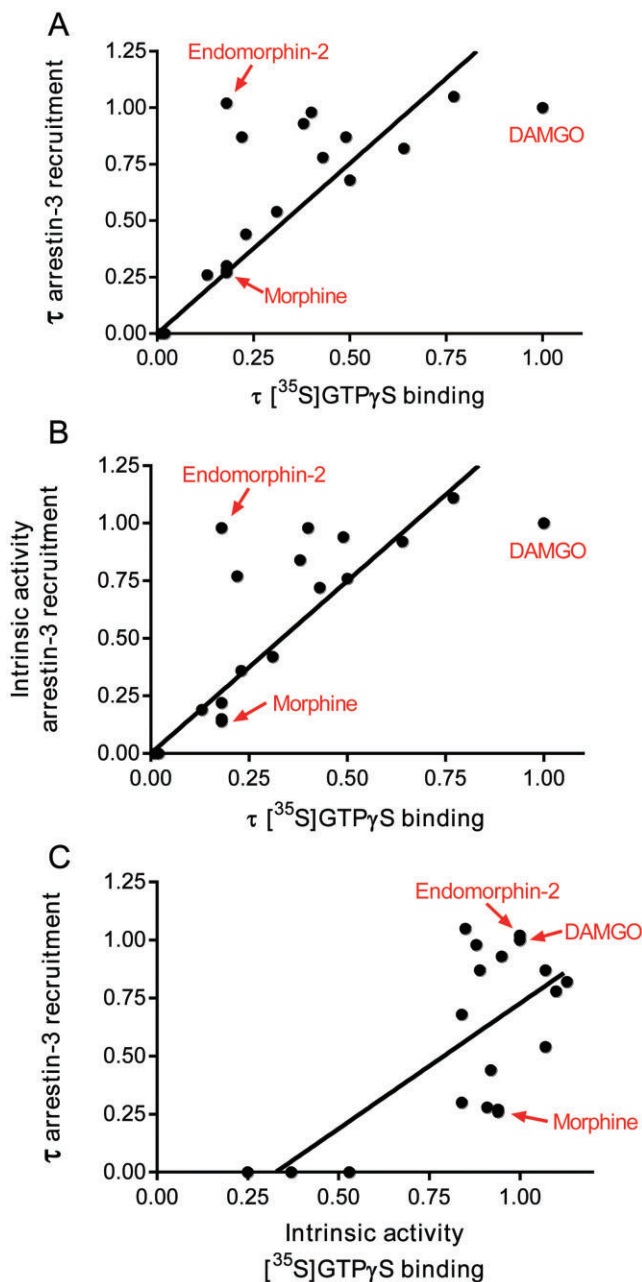
Figure 7

Correlation of efficacy values for [^{35}S]GTPγS and arrestin recruitment for a series of MOP receptor agonists. In each case, the data are expressed as a fraction of the value for DAMGO (taken as 1 in each case), and were subjected to linear regression. (A) Correlation of τ values with linear regression. (B) Correlation using intrinsic activity values for arrestin recruitment and τ values for [^{35}S]GTPγS binding, with linear regression. Note that the relationship when using intrinsic activity values for arrestin recruitment is essentially the same as when using τ values for this pathway as in A. (C) Correlation using intrinsic activity values for [^{35}S]GTPγS binding and τ values for arrestin recruitment, with linear regression. The use of intrinsic activity for G protein output now radically alters the relationship compared to graphs A and B. Note also that endomorphin-2 no longer appears as an arrestin-biased agonist, and in fact now it almost overlies DAMGO in the correlation.

(Zadina *et al.*, 1997) had not previously been identified as biased ligands. Further quantitative analysis of the data by a recently published method (Rajagopal *et al.*, 2011) indicated that endomorphin-2 was significantly biased towards arrestin recruitment over G protein activation, although it should be noted that a number of the agonists, such as etorphine and alfentanil, appeared to show at least some arrestin bias (see figure 8 in Rivero *et al.* 2012). The approach to quantify bias involves calculating the 'effective signalling' for a ligand (σ_{lig}) by the expression $\sigma_{lig} = \log(\tau_{lig}/\tau_{ref})$, where the τ value for a particular ligand for a signalling pathway (pathway 1) is compared to that of a reference, unbiased ('balanced') ligand in the same signalling pathway. This is repeated for another signalling output (pathway 2), allowing a bias factor (β) to be calculated (Rajagopal *et al.*, 2011):

$$\beta_{lig} = \frac{\{\sigma_{lig(pathway\ 1)} - \sigma_{lig(pathway\ 2)}\}}{\sqrt{2}} \quad (5)$$

The arrestin bias factor for endomorphin-2 was found to be −0.81 ± 0.18. A recent study where agonist-induced BRET between MOP receptors and G proteins, and MOP receptors and arrestin was investigated, did not identify endomorphin-2 as a biased agonist (Molinari *et al.*, 2010), but this may be because intrinsic activity, rather than intrinsic efficacy, was used in the correlation (this can be clearly seen from our data in Figure 7C). Careful inspection of the Molinari *et al.* (2010) data indicates that the EC$_{50}$ values for G protein activation for most MOP receptor agonists studied were significantly lower (~5–10-fold) than those for arrestin interaction (in line with a low or non-existent receptor reserve for the latter) – apart notably from endomorphin-2, where the potency for the two responses was the same. This strongly suggests that, relative to the other ligands tested, endomorphin-2 is biased towards arrestin recruitment over G protein activation. Further support for arrestin bias for endomorphin-2 comes from older studies, where the endomorphins were able to promote efficient internalization of MOP receptors (McConalogue *et al.*, 1999) yet were reported to be partial agonists in [^{35}S]GTPγS studies (Sim *et al.*, 1998). The finding that endomorphin-2 is an arrestin-biased ligand is of interest as it provides a lead to study the role of arrestin-dependent pathways in opioid receptor action (Figure 5), and also because there has been recent growing interest in ligands based on the structure of the endomorphins as efficient analgesics but with reduced tendency to induce dependence or respiratory depression (Wilson *et al.*, 2000; Varamini *et al.*, 2012).

Morphine an unbiased ligand?

Morphine was not identified as a biased ligand in our analysis, whereas it has been previously suggested to be a G protein-biased ligand (Borgland *et al.*, 2003; Raehal *et al.*, 2011). Indeed, early reports emphasized the inability of morphine to induce MOP receptor internalization while at the same time being able to activate G protein-dependent signalling pathways including K^{+} current activation and cyclic AMP inhibition (Whistler *et al.*, 1999; Zaki *et al.*, 2000). Can it therefore be that morphine is truly unbiased? An important
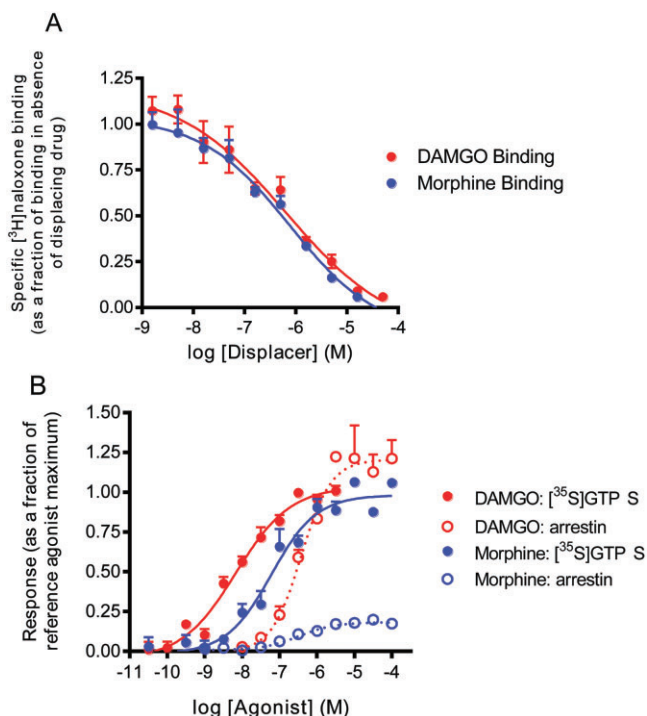
Figure 8

Comparison of the effects of DAMGO and morphine on ligand binding, [³⁵S]GTPγS binding and arrestin-3 recruitment. (A) DAMGO and morphine displaced [³H]naloxone from MOP receptors with similar potency. (B) Concentration–response curves for activation of [³⁵S]GTPγS binding and arrestin-3 recruitment for MOP receptors stably expressed in HEK293 cells. DAMGO was a full agonist in both assays, whereas morphine was a full agonist in the G protein assay but a weak partial agonist in the arrestin assay. The results in panel (A) indicate that differences between the DAMGO and morphine curves shown in (B) cannot be due to differences in affinity for MOP receptors.

point to grasp here is that just as two agonists producing the same response in a tissue can have different receptor reserves (Figure 2), so an agonist acting at one receptor subtype can produce two responses that have different receptor reserves, which has nothing to do with bias *per se*. For the MOP receptor agonists in general, the potency with which they activate G protein-coupled responses is greater (i.e. lower EC_{50}) than for regulatory responses (arrestin recruitment, phosphorylation, internalization and desensitization; Borgland *et al.*, 2003; McPherson *et al.*, 2010; Molinari *et al.*, 2010; Rivero *et al.*, 2012). Thus, the agonist receptor reserve (a reflection of efficacy) for G protein coupling is greater than that for the regulatory responses, meaning that for most agonists, the concentration response curve for G protein coupling lies to the left of that for, say, arrestin recruitment (Figure 8). For a high efficacy agonist such as DAMGO, it is possible to achieve maximum tissue responses for both assays. However, for a lower efficacy agonist like morphine, although it has sufficient efficacy to achieve a maximum response in the [³⁵S]GTPγS binding assay in the particular assay system that we employed, it does not have sufficient efficacy to achieve anything like the maximum response in

the arrestin recruitment assay. Visual inspection of the concentration–response curves, however, could lead one to conclude that morphine is biased towards the G protein response, as it appears such a weak agonist at the arrestin recruitment response relative to the G protein response (ironically our quantification of bias actually suggests that DAMGO is somewhat G protein biased; see Figure 7 in this review, also figure 8 in Rivero *et al.*, 2012). However, the important thing to compare is the *ratio* of intrinsic efficacy values (τ or whatever else is used) for the two different responses measured for each agonist. Using the τ values calculated previously (McPherson *et al.*, 2010), it can be seen that for many of the agonists investigated in that study, the $\tau_{G\ protein} : \tau_{arrestin}$ ratio is >20 for most agonists, including morphine. On this basis, we would not conclude that morphine is different from most of the other agonists tested, that is, morphine's actions in these assay systems can be explained by its relatively low efficacy for the two receptor responses measured. Further, we would predict that for an unbiased agonist with even lower efficacy for these responses than morphine, the agonist would be a partial one for [³⁵S]GTPγS binding but lack detectable agonist activity for arrestin-3 recruitment. This is exactly what is seen, for example, with buprenorphine, pentazocine and meptazinol (McPherson *et al.*, 2010).

Contrary to the findings described earlier, using MOP receptors expressed in AtT20 cells, Borgland *et al.* (2003) described morphine as an agonist with relatively high intrinsic efficacy for Ca^{2+} channel inhibition, which is a G protein-mediated response, yet very low efficacy for internalization in these cells. On this basis, morphine could be classified as a G protein-biased ligand, but this seems inconsistent with data from other systems, such as inhibition of the nerve-evoked contractions in rat vas deferens (considered to be a G protein-mediated response), where morphine is a very weak partial agonist/competitive antagonist compared to other MOP receptor agonists such as DAMGO (Ishii *et al.*, 1981; Smith and Rance, 1983). Furthermore, if morphine is a G protein-biased agonist, it might be expected to display effects similar to those observed with recently identified G protein-biased MOP receptor ligands (discussed below), such as reduced liability to induce tolerance and constipation (Lamb *et al.*, 2012; DeWire *et al.*, 2013), but this is certainly not the case.

The results from AtT20 cells (Borgland *et al.*, 2003) cannot be easily reconciled with our own data from HEK293 cells, unless one considers the possibility that the relative efficacies of ligands are subject to tissue-specific variation. For example, if morphine and DAMGO recruited distinct regulatory proteins to the G protein/arrestin signalling complex or even downstream of this, then if these protein partners were expressed at varying levels in tissues, this could consequently affect the relative efficacy values. This possibility is depicted in Figure 9, where the ability of morphine and DAMGO to signal is compared in two tissues or cell types. In the first tissue, DAMGO, due to its high intrinsic efficacy, is better able to promote coupling of MOP receptors to the downstream signal than morphine. In the second tissue, DAMGO also recruits an 'interacting protein', for example, a regulator of G protein signalling (RGS) protein (Psigfogeorgou *et al.*, 2011; Traynor, 2012), to the signalling complex (this protein may

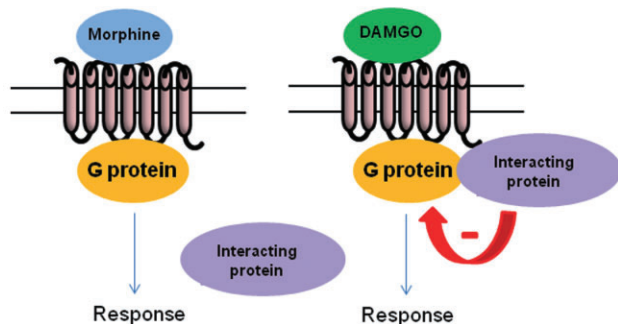## A Tissue 1
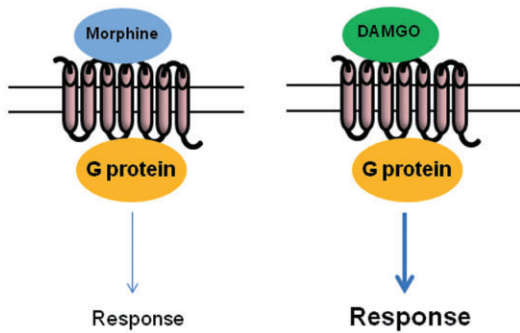
B Tissue 2

Figure 9

Possible mechanism to explain changes in relative intrinsic efficacy of MOP receptor agonists in different tissues/cell types. (A) In tissue 1, the high-efficacy agonist DAMGO is better able to promote coupling of MOP receptors via G protein to the downstream signal than the lower efficacy agonist morphine. (B) In tissue 2, which contains significant levels of an 'interacting protein', DAMGO but not morphine is able to promote recruitment of the 'interacting protein' to the signalling complex. Recruitment of the 'Interacting protein' dampens DAMGO-induced signalling, and consequently reduces the efficacy of DAMGO relative to morphine. DAMGO might have this effect either because it induces a conformation of the receptor-signalling complex more favourable to recruitment of the 'Interacting protein', or because the efficacy of morphine is not sufficient to recruit the 'Interacting protein'. In this example, an RGS protein (Psigfogeorgou et al., 2011; Traynor, 2012) could fulfil the role of an 'Interacting protein'. Furthermore, such dispatrties are more likely to occur if responses with different proximities to the receptor are compared. Thus, if G protein activation using [^{35}S]GTPγS was used in one assay, but the more distal readout of, say, an ion channel was used in another, then the latter could be subject to regulation by interacting proteins at more levels than the former response.

be absent or expressed at a much lower level in the first tissue), which may selectively dampen DAMGO-induced signalling, and consequently reduce the efficacy of DAMGO relative to morphine. DAMGO might have this effect either because it induces a conformation of the receptor-signalling complex more favourable to recruitment of the interacting protein, or because the efficacy of morphine is not sufficient to recruit the interacting protein.

In summary, the question of whether or not morphine is a biased agonist at MOP receptors remains a controversial

one, but given the clinical and experimental importance of this drug, it merits further study in different systems.

Efficacy, bias and the clinical use of opioids

What do pharmacological measures of efficacy and bias obtained in cells mean in terms of the clinical use of opioid drugs? This is a far from simple matter because the relative intrinsic efficacy of MOP receptor agonists obtained for complex responses such as analgesia will be a product not only of agonist action at MOP receptors and the immediate post-receptor environment but also pharmacokinetics and the selectivity of ligands for opioid receptor subtypes (Trescot et al., 2008). In addition, the type of pain being treated and the previous history of opioid medication in the patient will all feed into the overall analgesic effectiveness achieved (McQuay, 1999). It is of interest to note, however, that at the level of MOP receptor coupling, there is a large degree of variation in the efficacy for G protein activation, and indeed for other signalling outputs (Figure 7; also McPherson et al., 2010), yet clinicians using these drugs to induce analgesia would not recognize such differences in analgesic efficacy. Indeed, according to the British Pain Society's pamphlet 'Opioids for persistent pain: Good practice' (2010), buprenorphine, pentazocine and meperidine are each classified as 'strong opioids', whereas they exhibit relatively low efficacy in cell signalling assays. Accordingly, caution is required when extrapolating pharmacological efficacy from a cell-based test system to the possible in vivo effects of an opioid in a patient. Indeed, in spite of all the experimental research on opioids – often 3 or 4 papers per day on PubMed – morphine has been and remains the first choice for the treatment of severe pain such as in cancer (McQuay, 1999; Bennett et al., 2012). However, as noted previously (Hanks et al., 2001), morphine remains the first choice 'for reasons of familiarity, availability and cost rather than proven superiority'. Indeed, for all the work so far carried out to determine differences in MOP receptor agonist action, the adverse effects produced by the available ligands are much the same, with constipation and nausea being among the most troublesome (see Figure 5B). Even though buprenorphine is sometimes considered a safer opioid due to its 'ceiling effect' for inducing respiratory depression (Dahan et al., 2005), deaths due to buprenorphine-induced respiratory depression are still recorded (Mégarbane et al., 2006; Kim et al., 2012). Given the clinical importance of MOP receptor agonists, the sentiments expressed above should nevertheless not deter the experimenter from trying to understand the molecular actions of both established and recently synthesized MOP receptor ligands in terms of efficacy and bias, in the hope of obtaining better opioid drugs for the treatment of pain (Groer et al., 2007; Lamb et al., 2012).

Concluding remarks

What are the intracellular signalling pathways that mediate the therapeutic and/or adverse effects of opioid agonists

(Figure 5)? Can biased ligands at the MOP receptor be developed that selectively activate the former over the latter? What happens to these signalling pathways following prolonged MOP receptor activation? These remain central questions in the field of opioid receptor pharmacology. It might reasonably be concluded, for example, that G protein-biased MOP receptor ligands are desirable as these will provide effective analgesia with limited tolerance. Certainly, studies with the novel compound herkinorin, which has marked G protein bias and produces little if any arrestin recruitment (Groer *et al.*, 2007; Lamb *et al.*, 2012), suggest that such compounds have therapeutic potential. In addition, the recent identification of a G protein-biased MOP receptor ligand, TRV130 (DeWire *et al.*, 2013), which is effectively analgesic but produces less respiratory depression and constipation than morphine, is of great interest. On the other hand, ligands based on the structure of the endomorphins indicate that such ligands, which appear to be arrestin-biased, also have a desirable profile as they are analgesic yet produce little abuse potential or respiratory depression (Wilson *et al.*, 2000; Varamini *et al.*, 2012). What these studies really indicate is the need to fully understand the respective role of G proteins and arrestins in the action of agonists at MOP receptors, in producing both the therapeutic and the adverse effects of these drugs (Figure 5). However, this is not a trivial task because, for example, data with MOP receptor agonists from arrestin knockout mice have not provided simple conclusions (Raehal *et al.*, 2005; Dang *et al.*, 2011; Quillinan *et al.*, 2011; Kang *et al.*, 2012) perhaps because arrestin deletion results not only in the loss of that protein but also in other adaptive responses in the animal such as upregulation of JNK (Mittal *et al.*, 2012). Whatever the case, a key element in the development of new, potentially biased MOP receptor ligands will be the application of rigorous methods to determine pathway-dependent agonist efficacy and signalling so that reliable estimates of ligand bias can be obtained. This must be a priority in future studies.

Acknowledgements

Conflict of interest

The author declares no conflict of interest.

References

Alexander SP, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Bailey CP, Llorente J, Gabra BH, Smith FL, Dewey WL, Kelly E *et al.* (2009). Role of protein kinase C and mu-opioid receptor (MOP receptor) desensitization in tolerance to morphine in rat locus coeruleus neurons. Eur J Neurosci 29: 307–318.

Bassoni DL, Raab WJ, Achacoso PL, Loh CY, Wehrman TS (2012). Measurements of β-arrestin recruitment to activated seven transmembrane receptors using enzyme complementation. Methods Mol Biol 897: 181–203.

Bennett MI, Graham J, Schmidt-Hansen M, Prettyjohns M, Arnold S, Group GD (2012). Prescribing strong opioids for pain in adult palliative care: summary of NICE guidance. BMJ 344: e2806.

Black JW, Leff P (1983). Operational models of pharmacological agonism. Proc R Soc Lond B Biol Sci 220: 141–162.

Bohn L, Lefkowitz R, Gainetdinov R, Peppel K, Caron M, Lin F (1999). Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 286: 2495–2498.

Borgland S, Connor M, Osborne P, Furness J, Christie M (2003). Opioid agonists have different efficacy profiles for G protein activation, rapid desensitization, and endocytosis of mu-opioid receptors. J Biol Chem 278: 18776–18784.

Chan K, Brodsky M, Davis T, Franklin S, Inturrisi CE, Yoburn BC (1995). The effect of the irreversible mu-opioid receptor antagonist clocinnamox on morphine potency, receptor binding and receptor mRNA. Eur J Pharmacol 287: 135–143.

Chavkin C, Goldstein A (1982). Reduction in opiate receptor reserve in morphine tolerant guinea pig ilea. Life Sci 31: 1687–1690.

Chavkin C, Goldstein A (1984). Opioid receptor reserve in normal and morphine-tolerant guinea pig ileum myenteric plexus. Proc Natl Acad Sci U S A 81: 7253–7257.

Christie MJ, Williams JT, North RA (1987). Cellular mechanisms of opioid tolerance: studies in single brain neurons. Mol Pharmacol 32: 633–638.

Christopoulos A, El-Fakahany EE (1999). Qualitative and quantitative assessment of relative agonist efficacy. Biochem Pharmacol 58: 735–748.

Clarke WP, Berg KA (2010). Use of functional assays to detect and quantify functional selectivity. Drug Discov Today 7: e31–e36.

Clarke WP, Bond RA (1998). The elusive nature of intrinsic efficacy. Trends Pharmacol Sci 19: 270–276.

Colquhoun D (1998). Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. Br J Pharmacol 125: 924–947.

Cox EH, Kerbusch T, Van der Graaf PH, Danhof M (1998). Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram effect of synthetic opioids in the rat: correlation with the interaction at the mu-opioid receptor. J Pharmacol Exp Ther 284: 1095–1103.

Dahan A, Yassen A, Bijl H, Romberg R, Sarton E, Teppema L *et al.* (2005). Comparison of the respiratory effects of intravenous buprenorphine and fentanyl in humans and rats. Br J Anaesth 94: 825–834.

Dang VC, Chieng B, Azriel Y, Christie MJ (2011). Cellular morphine tolerance produced by βarrestin-2-dependent impairment of µ-opioid receptor resensitization. J Neurosci 31: 7122–7130.

Dewire SM, Yamashita DS, Rominger DH, Liu G, Cowan CL, Graczyk TM *et al.* (2013). A G protein-biased ligand at the µ-opioid

receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. J Pharmacol Exp Ther 344: 708–717.

Doll C, Pöll F, Peuker K, Loktev A, Glück L, Schulz S (2012). Deciphering μ-opioid receptor phosphorylation and dephosphorylation in HEK293 cells. Br J Pharmacol 167: 1259–1270.

Ehlert FJ (1985). The relationship between muscarinic receptor occupancy and adenylate cyclase inhibition in the rabbit myocardium. Mol Pharmacol 28: 410–421.

Ehlert FJ (2008). On the analysis of ligand-directed signaling at G protein-coupled receptors. Naunyn Schmiedebergs Arch Pharmacol 377: 549–577.

Ehlert FJ, Griffin MT, Suga H (2011). Analysis of functional responses at G protein-coupled receptors: estimation of relative affinity constants for the inactive receptor state. J Pharmacol Exp Ther 338: 658–670.

Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH, Medzihradsky F (1996). Characterization of opioid agonist efficacy in a C6 glioma cell line expressing the mu opioid receptor. J Pharmacol Exp Ther 278: 1121–1127.

Evans BA, Broxton N, Merlin J, Sato M, Hutchinson DS, Christopoulos A et al. (2011). Quantification of functional selectivity at the human α(1A)-adrenoceptor. Mol Pharmacol 79: 298–307.

Figueroa KW, Griffin MT, Ehlert FJ (2009). Selectivity of agonists for the active state of M1 to M4 muscarinic receptor subtypes. J Pharmacol Exp Ther 328: 331–342.

Frölich N, Dees C, Paetz C, Ren X, Lohse MJ, Nikolaev VO et al. (2011). Distinct pharmacological properties of morphine metabolites at G(i)-protein and β-arrestin signaling pathways activated by the human μ-opioid receptor. Biochem Pharmacol 81: 1248–1254.

Furchgott RF, Bursztyn P (1967). Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. Ann NY Acad Sci 144: 882–898.

Garrido M, Gubbens-Stibbe J, Tukker E, Cox E, von Frijtag J, Künzel D et al. (2000). Pharmacokinetic-pharmacodynamic analysis of the EEG effect of alfentanil in rats following beta-funaltrexamine-induced mu-opioid receptor 'knockdown' in vivo. Pharm Res 17: 653–659.

Griffin MT, Figueroa KW, Liller S, Ehlert FJ (2007). Estimation of agonist activity at G protein-coupled receptors: analysis of M2 muscarinic receptor signaling through Gi/o,Gs, and G15. J Pharmacol Exp Ther 321: 1193–1207.

Groer CE, Tidgewell K, Moyer RA, Harding WW, Rothman RB, Prisinzano TE et al. (2007). An opioid agonist that does not induce mu-opioid receptor – arrestin interactions or receptor internalization. Mol Pharmacol 71: 549–557.

Groer CE, Schmid CL, Jaeger AM, Bohn LM (2011). Agonist-directed interactions with specific beta-arrestins determine mu-opioid receptor trafficking, ubiquitination, and dephosphorylation. J Biol Chem 286: 31731–31741.

Haberstock-Debic H, Wein M, Barrot M, Colago EE, Rahman Z, Neve RL et al. (2003). Morphine acutely regulates opioid receptor trafficking selectively in dendrites of nucleus accumbens neurons. J Neurosci 23: 4324–4332.

Haberstock-Debic H, Kim KA, Yu YJ, von Zastrow M (2005). Morphine promotes rapid, arrestin-dependent endocytosis of mu-opioid receptors in striatal neurons. J Neurosci 25: 7847–7857.

Hall DA, Langmead CJ (2010). Matching models to data: a receptor pharmacologist's guide. Br J Pharmacol 161: 1276–1290.

Hanks GW, Conno F, Cherny N, Hanna M, Kalso E, McQuay HJ et al. (2001). Morphine and alternative opioids in cancer pain: the EAPC recommendations. Br J Cancer 84: 587–593.

Henry AG, Hislop JN, Grove J, Thorn K, Marsh M, von Zastrow M (2012). Regulation of endocytic clathrin dynamics by cargo ubiquitination. Dev Cell 23: 519–532.

Hull LC, Llorente J, Gabra BH, Smith FL, Kelly E, Bailey C et al. (2010). The effect of protein kinase C and G protein-coupled receptor kinase inhibition on tolerance induced by mu-opioid agonists of different efficacy. J Pharmacol Exp Ther 332: 1127–1135.

Ishii K, Yamamoto S, Muraki T, Kato R (1981). Partial agonistic action of morphine in the rat vas deferens. Jpn J Pharmacol 31: 905–909.

January B, Seibold A, Whaley B, Hipkin RW, Lin D, Schonbrunn A et al. (1997). beta2-adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. J Biol Chem 272: 23871–23879.

Just S, Illing S, Trester-Zedlitz M, Lau EK, Kotowski SJ, Miess E et al. (2013). Differentiation of opioid drug effects by hierarchical multi-site phosphorylation. Mol Pharmacol 83: 633–639.

Kahsai AW, Xiao K, Rajagopal S, Ahn S, Shukla AK, Sun J et al. (2011). Multiple ligand-specific conformations of the β2-adrenergic receptor. Nat Chem Biol 7: 692–700.

Kang M, Maguma HT, Smith TH, Ross GR, Dewey WL, Akbarali HI (2012). The role of β-arrestin2 in the mechanism of morphine tolerance in the mouse and guinea pig gastrointestinal tract. J Pharmacol Exp Ther 340: 567–576.

Kelly E, Bailey CP, Henderson G (2008). Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 153 (Suppl. 1): S379–S388.

Kenakin T (2013). New concepts in pharmacological efficacy at 7TM receptors. Br J Pharmacol 168: 554–575.

Kenakin T, Christopoulos A (2013). Signalling bias in new drug discovery: detection, quantification and therapeutic impact. Nat Rev Drug Discov 12: 205–216.

Kenakin T, Watson C, Muniz-Medina V, Christopoulos A, Novick S (2012). A simple method for quantifying functional selectivity and agonist bias. ACS Chem Neurosci 3: 193–203.

Kilpatrick LE, Holliday ND (2012). Dissecting the pharmacology of G protein-coupled receptor signaling complexes using bimolecular fluorescence complementation. Methods Mol Biol 897: 109–138.

Kim HK, Smiddy M, Hoffman RS, Nelson LS (2012). Buprenorphine may not be as safe as you think: a pediatric fatality from unintentional exposure. Pediatrics 130: e1700–e1703.

Lamb K, Tidgewell K, Simpson DS, Bohn LM, Prisinzano TE (2012). Antinociceptive effects of herkinorin, a MOP receptor agonist derived from salvinorin A in the formalin test in rats: new concepts in mu opioid receptor pharmacology: from a symposium on new concepts in mu-opioid pharmacology. Drug Alcohol Depend 121: 181–188.

Langemeijer EV, Verzijl D, Dekker SJ, Ijzerman AP (2013). Functional selectivity of adenosine A(1) receptor ligands? Purinergic Signal 9: 91–100.

Lau EK, Trester-Zedlitz M, Trinidad JC, Kotowski SJ, Krutchinsky AN, Burlingame AL et al. (2011). Quantitative encoding of the effect

of a partial agonist on individual opioid receptors by multisite phosphorylation and threshold detection. Sci Signal 4: ra52.

Luttrell LM, Gesty-Palmer D (2010). Beyond desensitization: physiological relevance of arrestin-dependent signaling. Pharmacol Rev 62: 305–330.

McConalogue K, Grady EF, Minnis J, Balestra B, Tonini M, Brecha NC et al. (1999). Activation and internalization of the mu-opioid receptor by the newly discovered endogenous agonists, endomorphin-1 and endomorphin-2. Neuroscience 90: 1051–1059.

McPherson J, Rivero G, Baptist M, Llorente J, Al-Sabah S, Krasel C et al. (2010). μ-Opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. Mol Pharmacol 78: 756–766.

McQuay H (1999). Opioids in pain management. Lancet 353: 2229–2232.

Madia PA, Dighe SV, Sirohi S, Walker EA, Yoburn BC (2009). Dosing protocol and analgesic efficacy determine opioid tolerance in the mouse. Psychopharmacol 207: 413–422.

Madia PA, Navani DM, Yoburn BC (2012). [(35)S]GTPγS binding and opioid tolerance and efficacy in mouse spinal cord. Pharmacol Biochem Behav 101: 155–165.

Mégarbane B, Hreiche R, Pirnay S, Marie N, Baud FJ (2006). Does high-dose buprenorphine cause respiratory depression?: possible mechanisms and therapeutic consequences. Toxicol Rev 25: 79–85.

Mjanger E, Yaksh TL (1991). Characteristics of dose-dependent antagonism by beta-funaltrexamine of the antinociceptive effects of intrathecal mu agonists. J Pharmacol Exp Ther 258: 544–550.

Mittal N, Tan M, Egbuta O, Desai N, Crawford C, Xie CW et al. (2012). Evidence that behavioral phenotypes of morphine in β-arr2–/– mice are due to the unmasking of JNK signaling. Neuropharmacol 37: 1953–1962.

Molinari P, Vezzi V, Sbraccia M, Grò C, Riitano D, Ambrosio C et al. (2010). Morphine-like opiates selectively antagonize receptor-arrestin interactions. J Biol Chem 285: 12522–12535.

Morgan MM, Christie MJ (2011). Analysis of opioid efficacy, tolerance, addiction and dependence from cell culture to human. Br J Pharmacol 164: 1322–1334.

Navratilova E, Waite S, Stropova D, Eaton MC, Alves ID, Hruby VJ et al. (2007). Quantitative evaluation of human delta opioid receptor desensitization using the operational model of drug action. Mol Pharmacol 71: 1416–1426.

Nickerson M (1956). Receptor occupancy and tissue response. Nature 178: 697–698.

Nickolls SA, Waterfield A, Williams RE, Kinloch RA (2011). Understanding the effect of different assay formats on agonist parameters: a study using the μ-opioid receptor. J Biomol Screen 16: 706–716.

Nijmeijer S, Vischer HF, Rosethorne EM, Charlton SJ, Leurs R (2012). Analysis of multiple Histamine H4 receptor compound classes uncovers Gαi protein- and β-Arrestin2-biased ligands. Mol Pharmacol 82: 1174–1182.

Osborne PB, Williams JT (1995). Characterization of acute homologous desensitization of mu-opioid receptor-induced currents in locus coeruleus neurones. Br J Pharmacol 115: 925–932.

Psigfogeorgou K, Terzi D, Papachatzaki MM, Varidaki A, Ferguson D, Gold SJ et al. (2011). A unique role of RGS9-2 in the striatum as a positive or negative regulator of opiate analgesia. J Neurosci 31: 5617–5624.

Quillinan N, Lau EK, Virk M, von Zastrow M, Williams JT (2011). Recovery from mu-opioid receptor desensitization after chronic treatment with morphine and methadone. J Neurosci 31: 4434–4443.

Raehal KM, Walker JK, Bohn LM (2005). Morphine side effects in beta-arrestin 2 knockout mice. J Pharmacol Exp Ther 314: 1195–1201.

Raehal KM, Schmid CL, Groer CE, Bohn LM (2011). Functional selectivity at the μ-opioid receptor: implications for understanding opioid analgesia and tolerance. Pharmacol Rev 63: 1001–1019.

Rajagopal S, Rajagopal K, Lefkowitz RJ (2010). Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nat Rev Drug Discov 9: 373–386.

Rajagopal S, Ahn S, Rominger DH, Gowen-MacDonald W, Lam CM, Dewire SM et al. (2011). Quantifying ligand bias at seven-transmembrane receptors. Mol Pharmacol 80: 367–377.

Rivero G, Llorente J, McPherson J, Cooke A, Mundell SJ, McArdle CA et al. (2012). Endomorphin-2: a biased agonist at the μ-opioid receptor. Mol Pharmacol 82: 178–188.

Rónai AZ, Al-Khrasani M, Benyhe S, Lengyel I, Kocsis L, Orosz G et al. (2006). Partial and full agonism in endomorphin derivatives: comparison by null and operational model. Peptides 27: 1507–1513.

Ruffolo RR (1982). Review important concepts of receptor theory. J Auton Pharmacol 2: 277–295.

Schulz S, Mayer D, Pfeiffer M, Stumm R, Koch T, Höllt V (2004). Morphine induces terminal micro-opioid receptor desensitization by sustained phosphorylation of serine-375. EMBO J 23: 3282–3289.

Selley DE, Sim LJ, Xiao R, Liu Q, Childers SR (1997). mu-Opioid receptor-stimulated guanosine-5'-O-(gamma-thio)-triphosphate binding in rat thalamus and cultured cell lines: signal transduction mechanisms underlying agonist efficacy. Mol Pharmacol 51: 87–96.

Selley DE, Liu Q, Childers SR (1998). Signal transduction correlates of mu opioid agonist intrinsic efficacy: receptor-stimulated [35S]GTP gamma S binding in mMOR-CHO cells and rat thalamus. J Pharmacol Exp Ther 285: 496–505.

Sim LJ, Liu Q, Childers SR, Selley DE (1998). Endomorphin-stimulated [35S]GTPgammaS binding in rat brain: evidence for partial agonist activity at mu-opioid receptors. J Neurochem 70: 1567–1576.

Smith CF, Rance MJ (1983). Opiate receptors in the rat vas deferens. Life Sci 33: 327–330.

Stallaert W, Christopoulos A, Bouvier M (2011). Ligand functional selectivity and quantitative pharmacology at G protein-coupled receptors. Expert Opin Drug Discov 6: 811–825.

Stephenson RP (1956). A modification of receptor theory. Br J Pharmacol Chemother 11: 379–393.

Strange PG (2008). Agonist binding, agonist affinity and agonist efficacy at G protein-coupled receptors. Br J Pharmacol 153: 1353–1363.

Traynor J (2012). μ-Opioid receptors and regulators of G protein signaling (RGS) proteins: from a symposium on new concepts in mu-opioid pharmacology. Drug Alcohol Depend 121: 173–180.

Trescot AM, Datta S, Lee M, Hansen H (2008). Opioid pharmacology. Pain Physician 11: S133–S153.

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H et al. (2007). Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320: 1–13.

Varamini P, Mansfeld FM, Blanchfield JT, Wyse BD, Smith MT, Toth I (2012). Lipo-endomorphin-1 derivatives with systemic activity against neuropathic pain without producing constipation. PLoS One 7: e41909.

Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E et al. (2013). Structural features for functional selectivity at serotonin receptors. Science 340: 615–619.

Walwyn W, Evans CJ, Hales TG (2007). Beta-arrestin2 and c-Src regulate the constitutive activity and recycling of mu opioid receptors in dorsal root ganglion neurons. J Neurosci 27: 5092–5104.

Whalen EJ, Rajagopal S, Lefkowitz RJ (2011). Therapeutic potential of β-arrestin- and G protein-biased agonists. Trends Mol Med 17: 126–139.

Whistler JL, Chuang HH, Chu P, Jan LY, von Zastrow M (1999). Functional dissociation of mu opioid receptor signaling and endocytosis: implications for the biology of opiate tolerance and addiction. Neuron 23: 737–746.

Williams JT, North RA (1984). Opiate-receptor interactions on single locus coeruleus neurones. Mol Pharmacol 26: 489–497.

Wilson AM, Soignier RD, Zadina JE, Kastin AJ, Nores WL, Olson RD et al. (2000). Dissociation of analgesic and rewarding effects of endomorphin-1 in rats. Peptides 21: 1871–1874.

Zadina JE, Hackler L, Ge LJ, Kastin AJ (1997). A potent and selective endogenous agonist for the mu-opiate receptor. Nature 386: 499–502.

Zaki PA, Keith DE, Brine GA, Carroll FI, Evans CJ (2000). Ligand-induced changes in surface mu-opioid receptor number: relationship to G protein activation? J Pharmacol Exp Ther 292: 1127–1134.

Zhang J, Ferguson SS, Barak LS, Bodduluri SR, Laporte SA, Law PY et al. (1998). Role for G protein-coupled receptor kinase in agonist-specific regulation of mu-opioid receptor responsiveness. Proc Natl Acad Sci U S A 95: 7157–7162.

Zheng H, Loh HH, Law PY (2008). Beta-arrestin-dependent mu-opioid receptor-activated extracellular signal-regulated kinases (ERKs) translocate to nucleus in contrast to G protein-dependent ERK activation. Mol Pharmacol 73: 178–190.