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GH: has no competing financial interests to declare.
YKL: has received consultancy fees from Syri Ltd.
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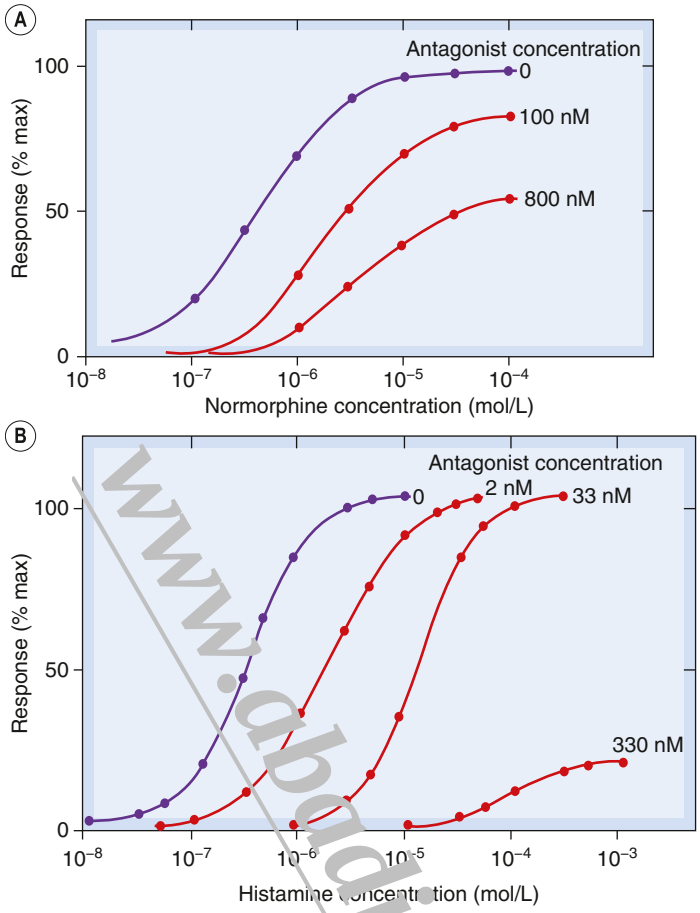

Fig. 2.6 Effects of irreversible competitive antagonists on agonist concentration–effect curves. (A) Rat brain neurons responding to the opioid agonist normorphine before and after being exposed to the irreversible competitive antagonist β-funaltrexamine for 30 minutes and then washed to remove the antagonist. Note the depression of the maximum response. (B) Responses of the guinea pig ileum to histamine before and after treatment with increasing concentrations of a receptor alkylating agent (GD121) for 5 minutes and then washed to remove the antagonist. Note the concentration–response curve is initially shifted to the right with no depression of the maximum response. (Panel [A] after Williams, J.T., North, R.A., 1984. Mol. Pharmacol. 26, 489–497; panel [B] after Nickerson, M., 1955. Nature 178, 696–697.)

and partial agonists lies in the relationship between receptor occupancy and response. In the experiment shown in Fig. 2.7 it was possible to estimate the affinity of the various drugs for the receptor, and hence (based on the theoretical model described later) to calculate the fraction of receptors occupied (known as *occupancy*) as a function of drug concentration. Plots of response as a function of occupancy for the different compounds are shown in Fig. 2.7B, showing that for partial agonists the response at a given level of occupancy is less than for full agonists. The weakest partial agonist, **tolazoline**, produces a barely detectable response even at 100% occupancy, and is usually classified as a *competitive antagonist* (see Ch. 15).

These differences can be expressed quantitatively in terms of *efficacy* (*e*), a parameter originally defined by Stephenson (1956) that describes the 'strength' of the agonist–receptor complex in evoking a response of the tissue. In the simple scheme shown in Fig. 2.1, efficacy describes the tendency of the drug–receptor complex to adopt the active (AR*), rather than the resting (AR), state. A drug with zero efficacy ($e = 0$) has no tendency to cause receptor activation, and causes no tissue response. A full agonist is a drug who efficacy[5] is sufficient that it produces a maximal response when less than 100% of receptors are occupied. A partial agonist has lower efficacy, such that 100% occupancy elicits only a submaximal response.

Subsequently it was appreciated that efficacy 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 (see Kelly, 2013). The tissue-dependent components of efficacy include the number of receptors that it expresses and the efficiency of coupling of receptor activation to the measured tissue response. The number of receptors expressed is especially relevant to the study of receptors in

[5]In Stephenson's formulation, efficacy is the reciprocal of the occupancy needed to produce a 50% maximal response, thus $e = 25$ implies that a 50% maximal response occurs at 4% occupancy. There is no theoretical upper limit to efficacy; indeed, some agonists are termed *super agonists* because they possess greater efficacy than the receptor's own endogenous agonist (e.g. dexmedetomidine, an α_2 adrenoceptor agonist with greater efficacy than that of either adrenaline or noradrenaline).

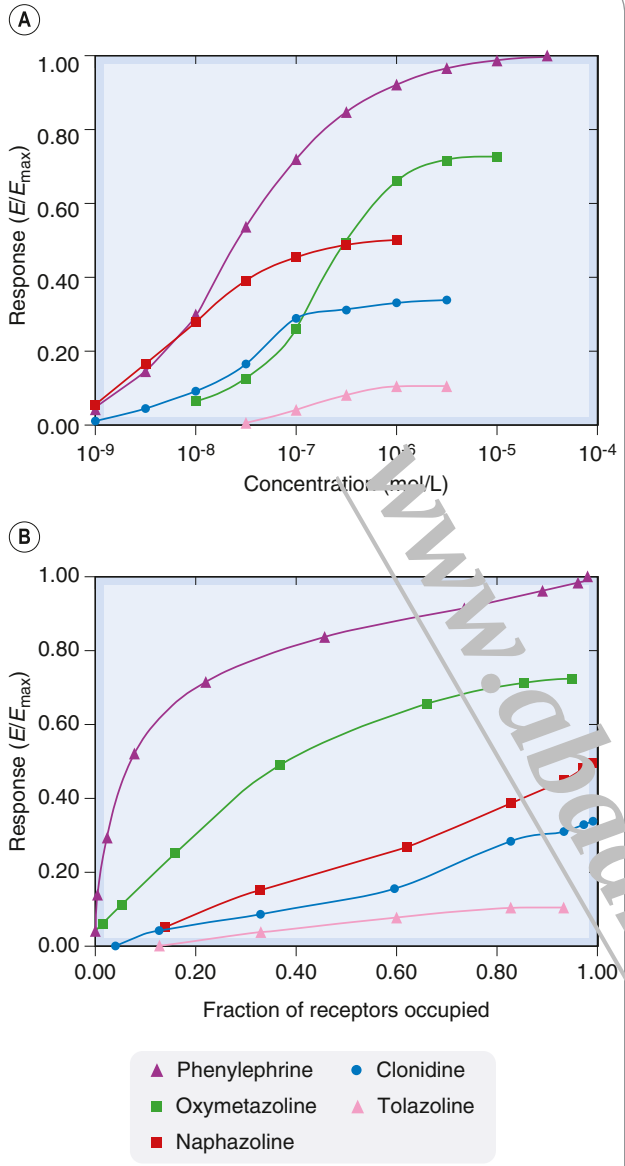

Fig. 2.7 Partial agonists. (A) Log concentration–effect curves for a series of α-adrenoceptor agonists causing contraction of an isolated strip of rabbit aorta. Phenylephrine is a full agonist. The others are partial agonists with different efficacies. The lower the efficacy of the drug the lower the maximum response and slope of the log concentration–response curve. (B) The relationship between response and receptor occupancy for the series. Note that the full agonist, phenylephrine, produces a near-maximal response when only about half the receptors are occupied, whereas partial agonists produce submaximal responses even when occupying all of the receptors. The efficacy of tolazoline is so low that it is classified as an α-adrenoceptor antagonist (see Ch. 15). In these experiments, receptor occupancy was not measured directly, but was calculated from pharmacological estimates of the equilibrium constants of the drugs. (Data from Ruffolo, R.R. Jr, et al., 1979. J. Pharmacol. Exp. Ther. 209, 429–436.)

recombinant expression systems when receptors are often very highly expressed and intermediate efficacy agonists then appear as full agonists. Across different cell types expressing the same receptor but at different densities, a given drug of intermediate efficacy may appear as a full agonist in one tissue (high level of receptor expression), a partial agonist in another (lower level of receptor expression) and even as an antagonist in another (very low level of receptor expression). The term 'partial agonist' is therefore only applicable when describing the action of a drug on a specific tissue or cell type.

For G protein–coupled receptors the elucidation of their X-ray crystal structures (described in Ch. 3) and the application of molecular dynamic simulations of drug binding are beginning to tease out the molecular basis of receptor activation and why some ligands are agonists and some are antagonists. For students starting to study pharmacology the simple theoretical two-state model described later provides a useful starting point.

PARTIAL AGONISTS AS ANTAGONISTS

In discussing the efficacy of partial agonists earlier, we considered the situation in which the tissue was exposed to only one drug, the partial agonist. What we should also consider is how the presence of a partial agonist would alter the response of a tissue to a higher efficacy agonist. This is depicted in Fig. 2.8 where it can be seen that the presence of the partial agonist induces some level of response dependent upon the concentration initially applied, but in addition because the partial agonist is competing with the full agonist for the receptors, it effectively acts as a competitive antagonist, shifting the concentration–response curve of the full agonist to the right. This is not just an obscure theoretical point but something which occurs in clinical practice. In the treatment of heroin users, buprenorphine, a weak partial agonist, not only acts as a weak opioid substitute but also acts as an antagonist and reduces the likelihood of overdose when users relapse and take heroin again (see Ch. 50).

CONSTITUTIVE RECEPTOR ACTIVATION AND INVERSE AGONISTS

Although we are accustomed to thinking that receptors are activated only when an agonist molecule is bound, there are examples (see De Ligt et al., 2000) where an appreciable level of activation (*constitutive activation*) may exist even when no ligand is present. These include receptors for benzodiazepines (see Ch. 45), cannabinoids (see Ch. 18), 5-hydroxytryptamine (see Ch. 16) and several other mediators. Furthermore, receptor mutations occur – either spontaneously, in some disease states (see Bond and Ijzerman, 2006), or experimentally created (see Ch. 4) – that result in appreciable constitutive activation. If a ligand reduces activity below the basal level of constitutive activation such drugs are known as *inverse agonists* (Fig. 2.9; see De Ligt et al., 2000) to distinguish them from *neutral antagonists*, which do not by themselves affect the level of activation. Inverse agonists can be regarded as drugs with negative efficacy, to distinguish them from agonists (positive efficacy) and neutral antagonists (zero efficacy). Neutral antagonists, by binding to the agonist binding site, will antagonise both agonists and inverse agonists. Inverse agonism was first observed at the benzodiazepine receptor (see Ch. 45) but such drugs are proconvulsive and thus not therapeutically useful! New examples of constitutively active receptors and inverse agonists are emerging with

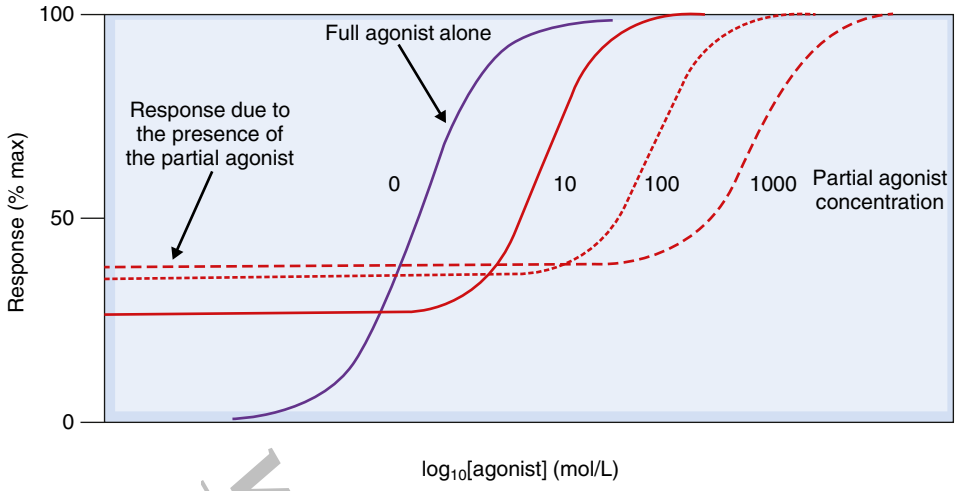

Fig. 2.8 Hypothetical concentration–response curves for a full agonist in the absence and presence of increasing concentrations of a partial agonist. The partial agonist will have agonist action and hence the initial response increases as the partial agonist concentration increases, reaching a maximum equal to the maximum response of the partial agonist. However, when the full agonist is added in the presence of the partial agonist its concentration–response curve is shifted to the right.

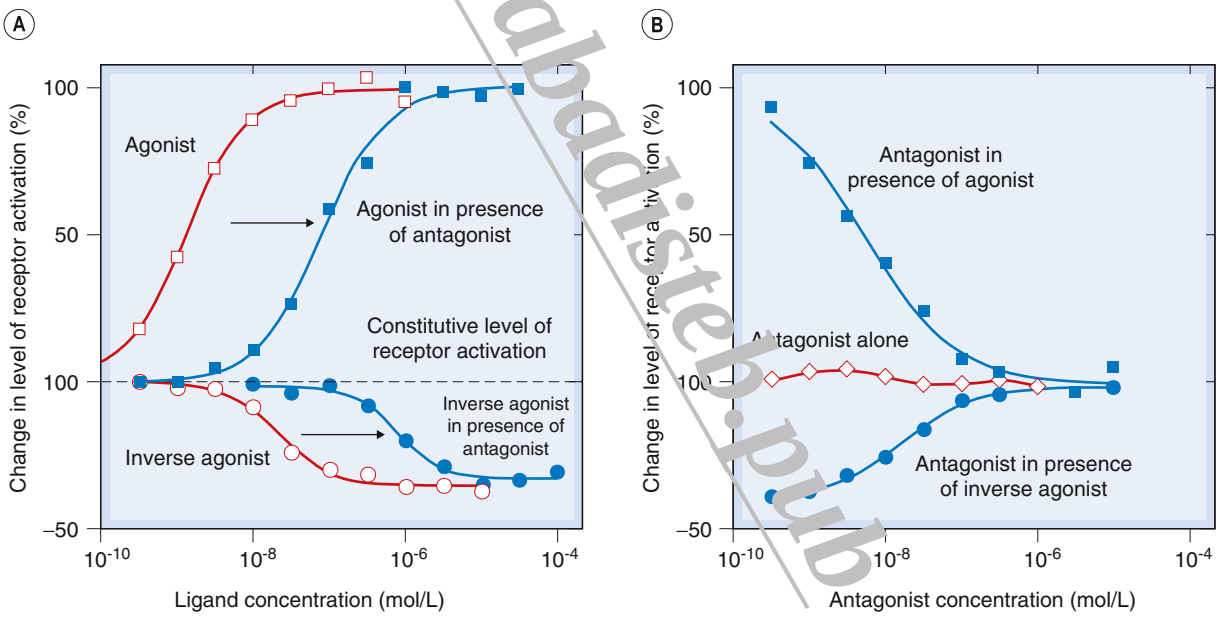

Fig. 2.9 Inverse agonism. The interaction of a competitive antagonist with normal and inverse agonists in a system that shows receptor activation in the absence of any added ligands (constitutive activation). (A) The degree of receptor activation (vertical scale) increases in the presence of an agonist *(open squares)* and decreases in the presence of an inverse agonist *(open circles)*. The addition of a competitive antagonist shifts both curves to the right *(closed symbols)*. (B) The antagonist on its own does not alter the level of constitutive activity *(open symbols)*, because it has equal affinity for the active and inactive states of the receptor. In the presence of an agonist *(closed squares)* or an inverse agonist *(closed circles)*, the antagonist restores the system towards the constitutive level of activity. These data were obtained with cloned human 5-hydroxytryptamine (5-HT) receptors expressed in a cell line. (Agonist, 5-carboxamidotryptamine; inverse agonist, spiperone; antagonist, WAY 100635; see Ch. 16 for information on 5-HT receptor pharmacology.) (Reproduced with permission from Newman-Tancredi, A., et al., 1997. Br. J. Pharmacol. 120, 737–739.)

increasing frequency (mainly among G protein–coupled receptors). **Pimavanserin**, an inverse agonist at the 5-HT_{2A} receptor, has recently been developed for the treatment of psychosis associated with Parkinson's disease (see Chs 40 and 47). It turns out that most of the receptor antagonists in clinical use are actually inverse agonists when tested in systems showing constitutive receptor activation. However, most receptors – like cats – show a preference for the inactive state, and for these there is no practical difference between a competitive antagonist and an inverse agonist, inverse

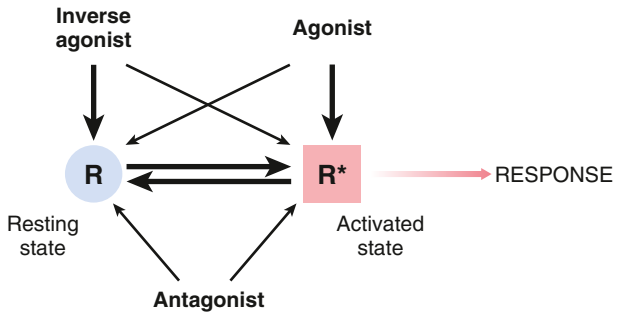

Fig. 2.10 The two-state model. The receptor is shown in two conformational states, *resting (R)* and *activated (R*)*, which exist in equilibrium. Normally, when no ligand is present, the equilibrium lies far to the left, and few receptors are found in the R* state. For constitutively active receptors, an appreciable proportion of receptors adopt the R* conformation in the absence of any ligand. Agonists have higher affinity for R* than for R, so shift the equilibrium towards R*. The greater the relative affinity for R* with respect to R, the greater the efficacy of the agonist. An inverse agonist has higher affinity for R than for R* and so shifts the equilibrium to the left. A *neutral antagonist* has equal affinity for R and R* so does not by itself affect the conformational equilibrium but reduces by competition the binding of other ligands.

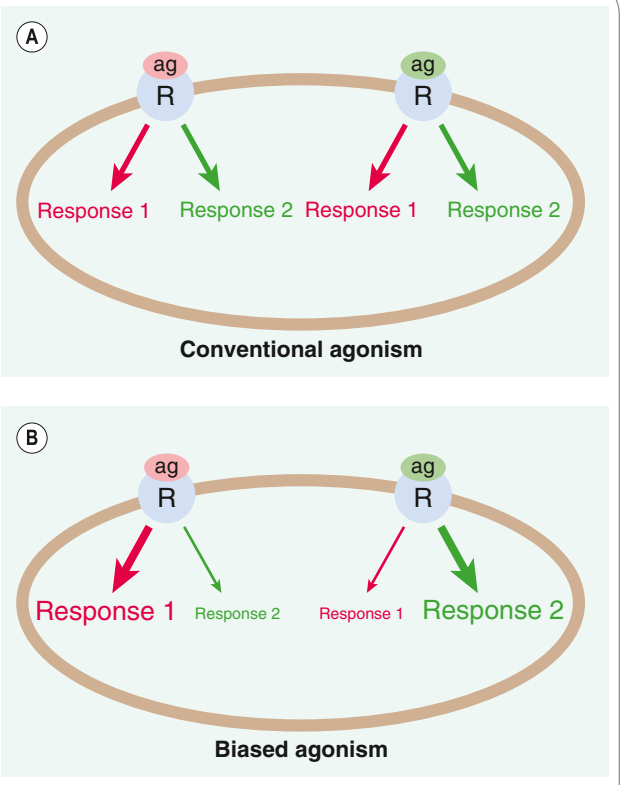

Fig. 2.11 Biased agonism. In (A), the receptor *(R)* is coupled to two intracellular responses – *response 1* and *response 2*. When different agonists indicated in *red* and *green* activate the receptor they evoke both responses in a similar manner. This is what we can consider as being conventional agonism. In (B), biased agonism is illustrated in which two agonists bind at the same site on the receptor yet the *red* agonist is better at evoking response 1 and the *green* agonist is better at evoking response 2.

agonism only being revealed if constitutive activation is observable.

The following section describes a simple model that explains full, partial and inverse agonism in terms of the relative affinity of different ligands for the resting and activated states of the receptor.

The two-state receptor model

As illustrated in Fig. 2.1, agonists and antagonists both bind to receptors, but only agonists activate them. How can we express this difference, and account for constitutive activity, in theoretical terms? The two-state model (Fig. 2.10) provides a simple but useful approach.

As shown in Fig. 2.1, we envisage that the occupied receptor can switch from its 'resting' (R) state to an activated (R*) state, R* being favoured by binding of an agonist but not an antagonist molecule.

As described earlier, receptors may show constitutive activation (i.e. the R* conformation can exist without any ligand being bound), so the added drug encounters an equilibrium mixture of R and R* (see Fig. 2.10). If it has a higher affinity for R* than for R, the drug will cause a shift of the equilibrium towards R* (i.e. it will promote activation and be classed as an agonist). If its preference for R* is very large, nearly all the occupied receptors will adopt the R* conformation and the drug will be a full agonist; if it shows only a modest degree of selectivity for R* (say 5- to 10-fold), a smaller proportion of occupied receptors will adopt the R* conformation and it will be a partial agonist; if it shows no preference, the prevailing R : R* equilibrium will not be disturbed and the drug will be a neutral antagonist (zero efficacy), whereas if it shows selectivity for R it will shift the equilibrium towards R and be an inverse agonist (negative efficacy). We can therefore think of efficacy as a property determined by the relative affinity of a ligand for R and R*, a formulation known as the *two-state model*, which is useful in that it puts a physical interpretation on the otherwise mysterious meaning of efficacy, as well as accounting for the existence of inverse agonists.

BIASED AGONISM

A major problem with the two-state model is that, as we now know, receptors are not actually restricted to two distinct states but have much greater conformational flexibility, so that there is more than one inactive and active conformation. The different conformations that they can adopt may be preferentially stabilised by different ligands, and may produce different functional effects by activating different signal transduction pathways (see Ch. 3).

Receptors that couple to second messenger systems (see Ch. 3) can couple to more than one intracellular effector pathway, giving rise to two or more simultaneous responses. One might expect that all agonists that activate the same receptor type would evoke the same array of responses (Fig. 2.11A). However, it has become apparent that different agonists can exhibit bias for the generation of one response over another even though they are acting through the same receptor (Fig. 2.11B), probably because they stabilise different activated states of the receptor (see Kelly, 2013). Agonist bias has become an important concept in pharmacology.

Redefining and attempting to measure agonist efficacy for such a multistate model is problematic, however, and requires a more complicated state transition model than the two-state model described previously. The errors, pitfalls and a possible way forward have been outlined by Kenakin and Christopoulos (2013).

ALLOSTERIC MODULATION

In addition to the agonist binding site (now referred to as the *orthosteric* binding site), to which competitive antagonists also bind, receptor proteins possess many other *(allosteric)* binding sites (see Ch. 3) through which drugs can influence receptor function in various ways, by increasing or decreasing the affinity of agonists for the agonist binding site, by modifying efficacy or by producing a response themselves (Fig. 2.12). Depending on the direction of the effect, the ligands may be allosteric antagonists or allosteric facilitators of the agonist effect, and the effect may be to alter the slope and maximum of the agonist log concentration–effect curve (see Fig. 2.12). This type of allosteric modulation of receptor function has attracted much attention recently and occurs at different types of receptors (see review by Changeux and Christopoulos, 2016). Well-known examples of allosteric facilitation include glycine at *N*-methyl-D-aspartate (NMDA) receptors (see Ch. 38), benzodiazepines at $GABA_A$ receptors (see Ch. 45) and **cinacalcet** at the Ca^{2+} receptor (see Ch. 36). One reason why allosteric modulation may be important to the pharmacologist and future drug development is that across families of receptors such as the muscarinic receptors (see Ch. 14) the orthosteric binding sites are very similar and it has proven difficult to develop selective agonists and antagonists for individual subtypes. The hope is that there will be greater variation in the allosteric sites and that receptor-selective allosteric ligands can be developed. Furthermore, positive allosteric modulators (PAMs) will exert their effects only on receptors that are being activated by endogenous ligands and have no effect on those that are not activated. This might provide a degree of selectivity (e.g. in potentiating spinal inhibition mediated by endogenous opioids, see Ch. 43) and a reduction in side effect profile.

BITOPIC AGONISTS

To further complicate the issue of drug–receptor interactions, some agonists may display a combination of orthosteric and allosteric actions at the same receptor, providing direct agonist and modulatory functions (see Volpato et al., 2020). These are termed bitopic agonists and we are likely to hear more about such drugs in the future.

OTHER FORMS OF DRUG ANTAGONISM

Other mechanisms can also account for inhibitory interactions between drugs.

The most important ones are:

- chemical antagonism
- pharmacokinetic antagonism
- block of receptor–response linkage
- physiological antagonism

CHEMICAL ANTAGONISM

Chemical antagonism refers to the uncommon situation where the two substances combine in solution; as a result, the effect of the active drug is lost. Examples include the use of chelating agents (e.g. **dimercaprol**) that bind to heavy metals and thus reduce their toxicity, and the use of the neutralising antibody **infliximab**, which has an anti-inflammatory action due to its ability to sequester the inflammatory cytokine tumour necrosis factor (TNF; see Ch. 17).

PHARMACOKINETIC ANTAGONISM

Pharmacokinetic antagonism describes the situation in which the 'antagonist' effectively reduces the concentration of the active drug at its site of action. This can happen in various ways. The rate of metabolic degradation of the active drug may be increased (e.g. the reduction of the anticoagulant effect of **warfarin** when an agent that accelerates its hepatic metabolism, such as **phenytoin**, is given; see Chs 10 and 58). Alternatively, the rate of absorption of the active drug from the gastrointestinal tract may be reduced, or the rate of renal excretion may be increased. Interactions of this sort, discussed in more detail in Chapter 58, are common and can be important in clinical practice.

BLOCK OF RECEPTOR–RESPONSE LINKAGE

Non-competitive antagonism describes the situation where the antagonist blocks at some point downstream from the agonist binding site on the receptor, and interrupts the chain of events that leads to the production of a response by the agonist. For example, **ketamine** enters the ion channel pore of the NMDA receptor (see Ch. 38) blocking it, thus preventing ion flux through the channels. Drugs such as **verapamil** and **nifedipine** prevent the influx of Ca^{2+} through the cell membrane (see Ch. 21) and thus non-selectively block the contraction of smooth muscle produced

Agonists, antagonists and efficacy

- Drugs acting on receptors may be *agonists* or *antagonists.*
- Agonists initiate changes in cell function, producing effects of various types; antagonists bind to receptors without initiating such changes.
- Agonist potency depends on two parameters: *affinity* (i.e. tendency to bind to receptors) and *efficacy* (i.e. ability, once bound, to initiate changes that lead to effects).
- For antagonists, efficacy is zero.
- *Full agonists* (which can produce maximal effects) have high efficacy; *partial agonists* (which can produce only submaximal effects) have intermediate efficacy.
- According to the two-state model, efficacy reflects the relative affinity of the compound for the resting and activated states of the receptor. Agonists show selectivity for the activated state; antagonists show no selectivity. This model, although helpful, fails to account for the complexity of agonist action.
- *Inverse agonists* show selectivity for the resting state of the receptor, this being of significance only in situations where the receptors show *constitutive activity.*
- *Allosteric modulators* bind to sites on the receptor other than the agonist binding site and can modify agonist activity.

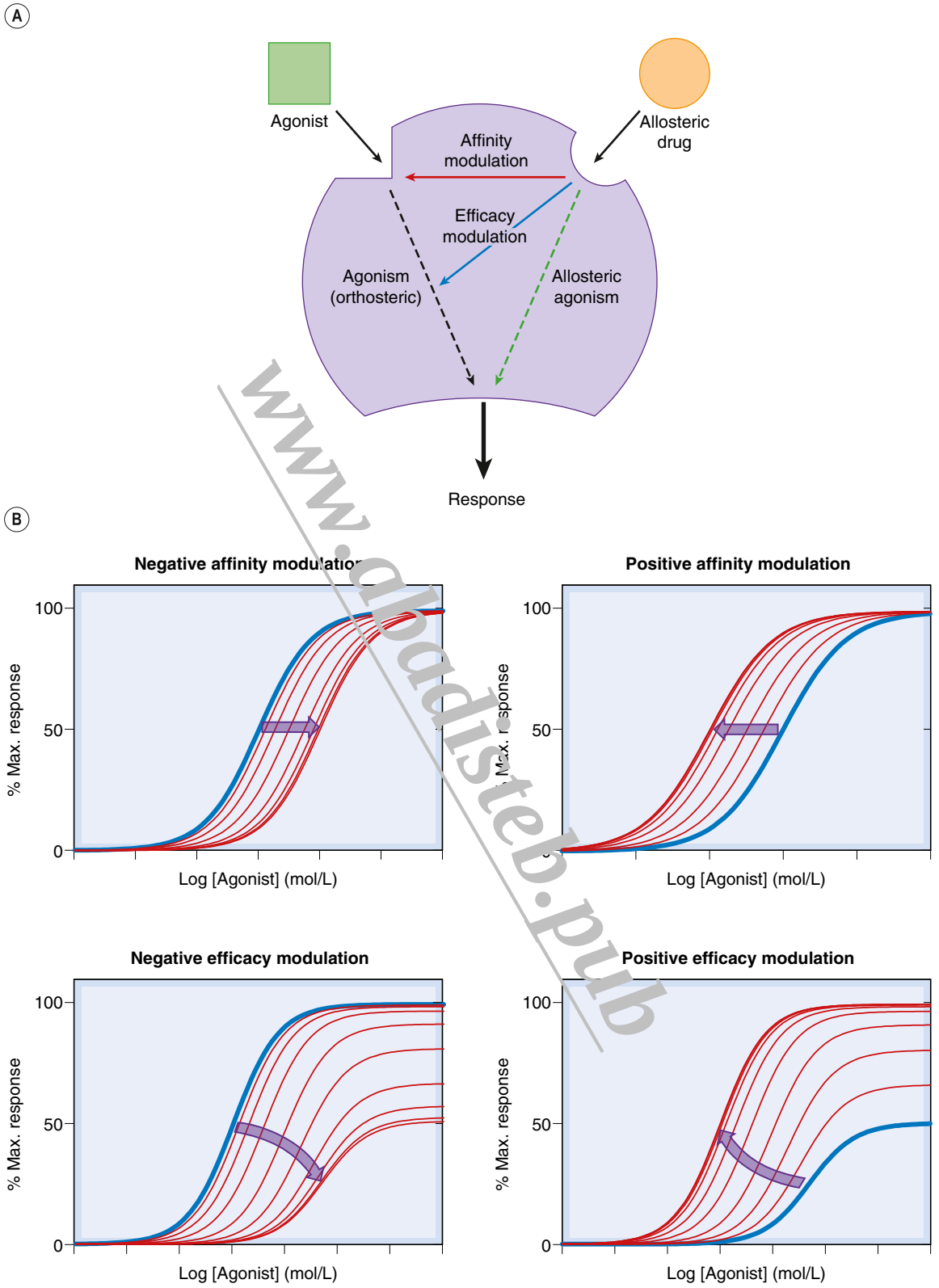

Fig. 2.12 Allosteric modulation. (A) Allosteric drugs bind at a separate site on the receptor to 'traditional' agonists (now often referred to as 'orthosteric' agonists). They can modify the activity of the receptor by (i) altering agonist affinity, (ii) altering agonist efficacy or (iii) directly evoking a response themselves. (B) Effects of affinity- and efficacy-modifying allosteric modulators on the concentration–effect curve of an agonist *(blue line)*. In the presence of the allosteric modulator the agonist concentration–effect curve *(now illustrated in red)* is shifted in a manner determined by the type of allosteric modulator until a maximum effect of the modulator is reached. (Panel [A] adapted with permission from Conn et al., 2009. Nat. Rev. Drug Discov. 8, 41–54; panel [B] courtesy Christopoulos, A.)