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Versatility of GPCR recognition by drugs: from biological implications to therapeutic relevance

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Most drugs acting on G-protein-coupled receptors (GPCRs) are classically defined as agonists, partial agonists or antagonists. This simplified classification seems sufficient to explain most of their therapeutic properties. The more recent description of inverse agonism has helped to revise theoretical models of GPCR function, but the therapeutic implications of the new concepts remain clearly restricted. Further complexity has arisen with demonstrations that a given receptor can adopt various conformations that support coupling with distinct G proteins. Because the related signaling pathways seem to be differentially affected by some ligands, the concept of 'functional selectivity' has been proposed, calling for a revision of the definitions of agonism and intrinsic efficacy. Evidence of complexity in G-protein coupling and examples of functional selectivity are accumulating, opening perspectives for drug development. Although such complexity should be regarded as an opportunity to gain pharmacological specificity, unraveling the physiological implications of these concepts is essential before their therapeutic relevance can be defined.

Introduction

For decades, the pharmacology of drugs acting at G-protein-coupled receptors (GPCRs) has been governed by concepts initially developed by Langley, Dale and Clark in the first half of the 20th century (reviewed in Ref. [1]). Indeed, their pioneering work established the first definitions of agonist and antagonist (see Glossary), which basically considered the ability of a ligand to promote or to impair the molecular conversion of the receptor in its active state. In this 'all or nothing' viewpoint, a comparison of active compounds is restricted to the measure of affinity and potency. Although it ignores the notion of molecular efficacy, this simplified classification seems sufficient to explain most of the therapeutic properties of drugs acting on GPCRs. Indeed, clinically active compounds are commonly regarded as selective activators or blockers of defined receptor subtypes. Thus, up until now, the principal challenge in drug development has been, in general, to design the most potent and selective compounds. Nevertheless, several biochemical and pharmacological studies have revealed the complexity of the functional interactions of drugs with GPCRs. The aim of this review is to provide an overview of these new concepts of pharmacodynamy and to discuss their relevance for future therapeutic perspectives.

Classical concepts of pharmacodynamy

In 1954, Ariens and de Groot [2] described the concept of 'partial agonism', whereby distinct agonists of a given receptor might differ in the amplitude of the functional response that they elicit after maximal receptor occupancy. The characterization of this behavior directly led to definition of the 'intrinsic activity' as a key parameter influencing the ability of a drug to induce the functional response on interaction with the receptor. Intrinsic activity is completely independent of affinity and potency, and partial agonists should not be regarded as weak competitors with modest clinical efficacy. Accumulating data indicate that partial agonism contributes to the therapeutic efficacy of several drugs (e.g. buspirone, buprenorphine, pindolol and salbutamol). Indeed, partial agonism endows the drug with the dual features of agonism and antagonism, depending on the presence of another active chemical (in particular, the endogenous transmitter). As a consequence, these drugs are frequently recognized as stabilizers of cell communication, enhancing deficient systems while simultaneously blocking excessive activity [3].

The clinical relevance of such a multifaceted pharmacological profile is exemplified by the antipsychotic drug aripiprazole, which achieves a subtle control of mesocortical and mesolimbic dopamine transmission [4], and by buprenorphine, a partial agonist of opioid receptors that causes less addiction and dependence as compared with full agonists [5]. In addition, it has been suggested that partial agonism prevents the adaptive regulatory mechanisms that frequently develop after repeated exposure to potent full agonists or antagonists [6].

Constitutive activity, inverse and protean agonists

Constitutive activity and inverse agonism

More recently, the high density of recombinant GPCRs that are expressed in engineered systems revealed that

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Glossary

Agonist: an orthosteric ligand with selective affinity for the active conformation of the receptor. Through stabilization of this active conformation, an agonist promotes the induction of related intracellular responses. Full agonists show high intrinsic efficacy.

Allosteric ligand (modulator): a ligand that interacts with the receptor at a site distinct from the site recognized by the endogenous ligand (transmitter or hormone). On binding, an allosteric ligand can alter the constitutive activity of the receptor and qualitatively or quantitatively influence the activity of orthosteric ligands in a non-competitive manner.

Antagonist (neutral antagonist): an antagonism profile defines a competitor that does not discriminate between the different active and inactive conformations of the receptor. Therefore, binding of an antagonist does not affect the spontaneous equilibrium that defines the constitutive activity of the receptor in a given system. When competing with agonists or inverse agonists, an antagonist impairs responses and brings the system back to its constitutive activity.

Collateral efficacy: this concept is directly related to the ability of ligands acting on a receptor to trigger multiple independent responses. The collateral efficacy reflects the capacity of a given ligand to trigger a subset of these responses without causing activation of all of the signals commonly induced by the endogenous agonist of the receptor. In particular, some ligands with collateral efficacy have the capacity to induce receptor desensitization without activating classical signaling cascades.

Functional selectivity: several receptors independently activate distinct functional responses. The functional selectivity of a ligand refers to its property to influence these responses differentially.

Intrinsic activity (α): this term refers to the relative amplitude of the biochemical or physiological response induced by a ligand when present at a concentration ensuring maximal receptor occupancy. Reference full agonists have an intrinsic activity value of 1.

Intrinsic efficacy (ε): this term classically defines the capacity of a ligand to produce a receptor 'stimulus'. At the molecular level, it describes the ability of the ligand to stabilize the active conformation of the receptor. Because several receptors independently activate different functional responses through distinct active conformations, a given ligand can show multiple intrinsic efficacies, depending on the nature of the response examined.

Inverse agonist: an orthosteric ligand with selective affinity for the inactive conformation of the receptor. Through stabilization of this inactive conformation, an inverse agonist prevents the induction of related intracellular responses. A key characteristic of inverse agonists is their ability to inhibit the response associated with the constitutive activity of the receptor. Full inverse agonists show low intrinsic efficacy.

Orthosteric ligand (modulator): a ligand that interacts with the receptor at a site that corresponds to (or overlaps with) the site recognized by the endogenous ligand (transmitter or hormone). Orthosteric ligands therefore compete with the endogenous ligand for the receptor and behave as agonists, inverse agonists or antagonists.

Partial agonist or partial inverse agonist: an orthosteric ligand that interacts with both the active and the inactive conformations of the receptor. Partial agonists and partial inverse agonists favor stabilization of the active and inactive conformations, respectively.

Permissive antagonism: this concept refers to the capacity of an allosteric ligand to alter differentially independent responses induced by an agonist on a given receptor. Thus, a permissive allosteric ligand can block some of the responses to this agonist, while leaving other responses to the same agonists unaffected. By contrast, a non-permissive allosteric ligand interferes with all responses associated with stimulation of the receptor.

Probe dependency: this term describes the capacity of an allosteric ligand to influence differentially the functional responses to distinct orthosteric ligands. This concept is related to the existence of functional selectivity of the latter ligands. As a result, the modulation obtained with the allosteric ligand will differ with respect to the nature of the reference orthosteric ligand tested.

Protean agonist: an orthosteric ligand that behaves as either a partial agonist or a partial inverse agonist, depending on the constitutive activity of the receptor in a given system. Thus, in systems where the constitutive activity is high, protean agonists stabilize the inactive conformation; in models with low constitutive activity, by contrast, they stabilize the active conformation.

these membrane proteins have constitutive activity. The constitutive activity of GPCRs is not only restricted to artificial models, but is also likely to exist in physiological systems [7]. The recent observation that native 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors show constitutive activity in rat hippocampal membranes [8] corroborates the hypothesis that the anxiolytic activity of partial agonists of 5-HT_{1A} such as buspirone could involve subtle modulation of the putatively altered spontaneous activity of these receptors [9].

In addition, several mutations in GPCRs result in enhanced constitutive activity [10], leading to inherited human diseases such as adrenocorticotropin resistance and hypocalciuric hypercalcemia [11]. Because significant activity of these receptors can be detected in the absence of agonist, some drugs, which had commonly been considered as antagonists, were subsequently found to reduce this activity. These drugs were assigned negative values of intrinsic activity, leading to the concept of 'inverse agonism' [12]. Initially considered as singular tools for *in vitro* pharmacodynamic studies, drugs endowed with an inverse agonism profile have now been established to have a different pharmacological potential from that of the so-called 'neutral antagonists' [13]. In particular, as mentioned earlier for partial agonists, it is likely that inverse agonists will have a distinct effect on receptor regulation [14].

Conformational model of GPCRs

The identification of partial agonists and inverse agonists indicated that the previous classification of GPCR ligands into pure agonists or pure antagonists is oversimplified and incomplete. It is now considered that full agonism and full inverse agonism constitute the extreme endpoints of a continuum that characterizes the functional properties of orthosteric ligands (Figure 1). Progressive revision of the theoretical model suggesting that interaction with a drug triggers GPCR activation has led to the proposal of the 'conformational model'.

In this model, the receptor constantly oscillates between an active and an inactive conformation. So-called agonists bind with high affinity to the active conformation, which they stabilize, whereas inverse agonists favor stabilization of an inactive conformation [15]. The intrinsic efficacy is viewed here as a geometric parameter that characterizes the GPCR ligand, referring to its dominant interaction with the active or the inactive conformation [16]. In the conformational model, neutral antagonists are competing ligands that do not discriminate between the active and inactive conformation. As a consequence, on their own these antagonists do not favor any of the conformations of the receptor, but have the capacity to interfere with the activity of other ligands.

Although the conformational model provides clues to the comprehensive classification of chemical entities interacting with GPCRs, its implications for clinical pharmacology have been frequently underestimated. Clearly, the use of inverse agonists that stabilize the inactive conformation instead of neutral antagonists is certainly preferable for the treatment of human diseases associated with GPCR-activating mutations [14]. In addition, more recent studies have found that several native receptors, such as 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors [8,17], melanocortin receptors [18] and histamine H_3 receptors [19], show noticeable constitutive activity in physiological conditions, suggesting that further consideration should be given to the potential therapeutic benefit of inverse agonists.

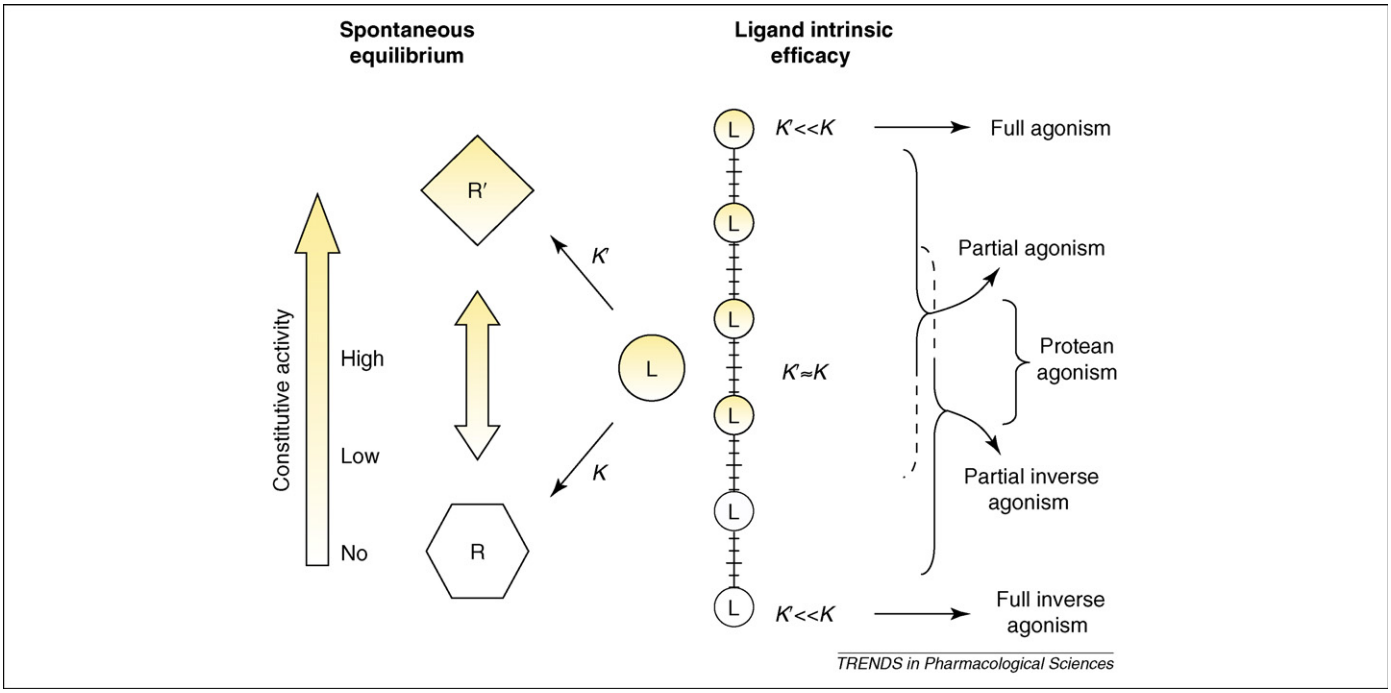

Figure 1. Constitutive activity, intrinsic efficacy and profiles of interacting ligands. Left, in the absence of ligand, an equilibrium spontaneously establishes between the active and inactive conformations of the receptor (R and R′, respectively). The position of this equilibrium determines the constitutive activity, which is influenced by several extrinsic factors (e.g. cell environment, interacting proteins and allosteric ligands). Right, a ligand interacting with the receptor might influence this equilibrium through differential stabilization of these conformations. The profile of the ligand is therefore defined by its intrinsic efficacy, which can be viewed as the ratio of affinity for these two conformations (K and K'). Ligands showing predominant affinity for the active conformation ($K'/K > 1$) should be viewed as agonists, whereas those stabilizing the inactive conformation are considered as inverse agonists. Full agonists and full inverse agonists are clearly positioned at the endpoints of this intrinsic efficacy spectrum, whereas ligands showing affinity for both conformations are considered as partial agonists or partial inverse agonists. There is overlap between the ranges of intrinsic efficacy defining partial agonism and partial inverse agonism, which reflects the effect of the level of constitutive activity of the receptor. Thus, in models where the constitutive activity is low, several ligands with intermediate intrinsic efficacy values will behave as partial agonists. In models with high constitutive activity, by contrast, the same ligands could displace the equilibrium in favor of the inactive conformation and therefore behave as partial inverse agonists. The modest values of intrinsic efficacy of these ligands endows them with the dual features of agonist and inverse agonist, and they are therefore defined as 'protean agonists'. Note that most receptors can probably adopt various active and inactive conformations. For simplification, the example shown considers the existence of only one active and one inactive conformation.

Protean agonism

Although partial agonism (and partial inverse agonism) refers to the property of ligands to interact with both the inactive and the active conformations of the receptor, partial agonists are commonly viewed as drugs that have a positive effect on cell response. Depending on their intrinsic efficacy and the constitutive activity of the receptor in a given model, however, these ligands can be predicted to show agonist or inverse agonist properties (Figure 1). Thus, the recently described concept of 'protean agonism' proposes that drugs endowed with modest intrinsic efficacy promote stabilization of the active conformation when constitutive activity is low, but stabilize the inactive conformation when constitutive activity is high. Extending the concept of transmission stabilizers of partial agonists (see earlier), protean agonists should constitute useful drugs for the treatment of disorders characterized by GPCR dysfunction. For example, drugs with appropriate intrinsic efficacy would reduce or enhance abnormal receptor function and modestly influence *per se* healthy systems.

This concept has been nicely demonstrated by Ganguli *et al.* [20], who have shown that introducing selected mutations in the secretin receptor causes an increase in constitutive activity and switches natural peptide ligands from agonists to inverse agonists. In addition, the interaction of levomedetomidine with α_{2A} adrenoceptors has been shown to elicit either positive or negative responses when examined in distinct models characterized by different levels of receptor G-protein pre-coupling [21]. Although several ligands showing inverse or protean agonist behavior are available [22,23], better characterization of deregulated receptor constitutive activity in diverse pathologies should help to define the potential therapeutic benefit of these drugs. Of note, region-specific editing of the 5-HT_{2C} receptor in the brain has been recently proposed to affect the extent of constitutive activity, which could potentially contribute to the antipsychotic properties of inverse agonists of 5-HT_{2C} [24].

In addition to situations involving alteration of the receptor structure (e.g. mutation, editing and polymorphism), constitutive activity is likely to be influenced by factors in the local environment such as the availability of interacting G proteins. Indeed, opposite responses to proxyphan, a protean ligand of the histamine H_3 receptor, have been observed *in vivo* in a comparison of its influence on the sleep–wake cycle in different animal species [25]. Lastly, particular interest should be given to the properties of protean ligands in circumstances where constitutive GPCR responses are potentially altered by dysfunction of intracellular signaling partners such as regulators of G-protein signaling [26].

Further complexity: multiple functional receptor conformations

Functional selectivity of GPCRs

In addition to demonstrating the existence of constitutive activity, extensive characterization of the properties of receptors expressed in recombinant systems has revealed that it is possible for a given receptor subtype to trigger intracellular signals through distinct G proteins. This finding has inevitably raised questions regarding both the physiological and the pharmacological significance of such promiscuous behavior of these receptors. Experimental data have clearly demonstrated, however, that in a given system the coupling efficiencies and specificities are tightly controlled by extrinsic factors [27]. Thus, such complexity in G-protein coupling should be regarded as an opportunity to gain therapeutic specificity by designing discriminating pharmacological tools that can dictate the receptor signaling. Accordingly, several synthetic agonists or antagonists have been shown to control differentially the activation of individual G proteins.

Initially termed 'agonist-selective trafficking of receptor signaling' [28], this concept is now best described as the 'functional selectivity' of a GPCR ligand [29], suggestive of its potential importance for future drug development. Indeed, this exciting behavior of some GPCRs agonists is now frequently thought to participate in their atypical properties. For example, recent *in vitro* data indicate that the putative functional selectivity of the antipsychotic drug aripiprazole at the dopamine D2 receptor might explain its therapeutic efficacy [30]. Similarly, the hallucinogen action of selected psychoactive substances interacting with 5-HT receptors is likely to be associated with the differential activation of a subset of signaling cascades [31].

The concept of multiplicity of coupling adds another degree of complexity when trying to define the pharmacodynamic parameters of GPCR ligands. For example, assuming its independent coupling with distinct G proteins, a given drug will possess distinct intrinsic efficacies because it can simultaneously act as a full or partial agonist, an antagonist, or a full or partial inverse agonist at the same receptor subtype when considering the different signaling cascades. At the molecular level, this observation is best explained by considering that several active conformations of the receptor exist that differ in their coupling efficiencies with distinct G proteins [32,33] (Box 1). Merging this concept with the conformational model mentioned earlier, the functional response will depend on the ability of an orthosteric drug to modify the equilibrium between these multiple conformations. Thus, these ligands influence either positively or negatively the coupling between a receptor and distinct G proteins, and their intrinsic efficacy will rely on the nature of the response examined.

Optimizing functional selectivity

In the past few years, evidence for the complexity of G-protein coupling has accumulated for numerous GPCRs. Simultaneously, detailed characterization of several GPCR-interacting ligands has shown that it is possible to manipulate the related cell signals specifically. Thus, in addition to drugs with selective agonist properties, ligands with a mixed agonist and antagonist or inverse agonist profile have been described [34,35]. The physiological implication of these observations, however, frequently remains obscure; consequently, the pharmacological relevance of these drugs seems rather speculative. Nevertheless, optimizing functional selectivity would contribute to the development of new therapeutic approaches, as has been recently suggested for selected drugs acting on β_2 adrenoceptors.

During heart failure, the persistent increase in adrenergic tone leads to molecular adaptations consisting of a downregulation of β_1 adrenoceptors and a switch of β_2 adrenoceptor coupling from G_s to G_i proteins [36]. Although the rational treatment of several cardiovascular diseases involves β_1 adrenoceptors antagonists, which are expected to normalize cardiac functions owing to their negative inotropic and chronotropic effects, the treatment of individuals with chronically failing heart also requires restoration of the contractile response, for which selective activation of β_2 adrenoceptors should certainly be considered. Although the adaptive switch to predominant G_i coupling contributes to antiapoptotic benefits, however, it also confers negative inotropic properties to β_2 adrenoceptor agonists. It is therefore important to select β_2 adrenoceptor ligands that will bring an appropriate balance of G_s and G_i activation to combine the inotropic and antiapoptotic responses [37]. Detailed characterization of the properties of commonly used adrenergic ligands could uncover their previously unexpected functional selectivity and thereby justify their privileged use as therapeutics [38,39].

In addition, giving preference to functionally selective ligands could also help to minimize putative side-effects associated with the complexity of cell signals activated by a single receptor subtype, as exemplified by newly developed ligands of the histamine H_1 receptor. Stimulation of this receptor elicits activation of both phospholipase C and adenylyl cyclase, indicative of a dual coupling with G_q and G_s proteins [40]. These signaling cascades are associated, respectively, with histamine-mediated allergic responses and hyperalgesia, and with a modulatory influence on psychiatric functions through regulation of catecholamine synthesis [34,41]. In contrast to histamine, the high-affinity ligand Trans-PAT has been shown to behave as a competitive antagonist or full agonist when these functional responses are examined separately. The possibility of promoting specific activation of adenylyl cyclase holds promise for identifying psychoactive histamine H1 ligands devoid of peripheral side-effects, such as bronchoconstriction, hypotension and edema [34].

Dissociating functional responses and receptor regulation

Another consequence of the complexity of signals associated with a single receptor subtype is that the regulation of the receptor could be induced independently of the activation of its commonly examined signaling pathways [42]. Best documented is the different profile of μ opioid receptor phosphorylation, desensitization and internalization observed after exposure to either morphine or DAMGO. Although the mechanisms remain largely debated, it is

assumed that these potent agonists stabilize distinct conformations of the receptor [43]. Considering the implication of opioid receptor regulation in the development of tolerance and dependence, achieving better control of opioid receptor trafficking could certainly enhance the pharmacological efficacy and safety of opioid ligands. Indeed, the possibility of pharmacologically manipulating receptor regulation independent of the functional response expected from common agonists constitutes a promising new therapeutic approach.

For example, recent studies have highlighted the potential therapeutic benefit of drugs with high efficacy but low capacity to induce desensitization, which would permit maintenance of the drug response during prolonged uses [44]. Conversely, some ligands have been shown to induce receptor internalization despite an absence of the commonly examined functional response [45,46]. Such behavior is best explained by considering that these putative antagonists stabilize a desensitization-prone conformation (collateral efficacy) that does not necessarily induce additional signals [47].

A possible therapeutic application of such ligands lies in tumor therapies. Overexpressed GPCRs have been identified in cancer tissues [48]; thus, newly designed ligands would aim at promoting their desensitization without activating the signaling pathways putatively associated with tumor progression, invasion and metastasis. Similarly, GPCRs have been implicated as entry points for viral particles in target cells, and specific antagonists of these receptors have been proposed as antiviral agents [49]. As an alternative, those drugs with collateral efficacy could contribute to impair viral entry while preserving physiological cell functions.

Lastly, several human diseases have been associated with abnormal regulation of selected GPCRs. The clinical efficacy of drugs in depressive disorders has been frequently shown to reflect the ability of the drugs to restore GPCR homeostasis through downregulation, whereas excessive activation of these receptors results in undesirable side-effects. Recent studies have reported on the internalization of 5-HT_{2A} receptors induced by antagonists – an effect that is likely to be correlated with their unexpected clinical efficacy [50].

Allosteric ligands

Integrating recent findings with earlier concepts of pharmacodynamy, the conformational model of GPCRs, which explains regulation of the activation of multiple G proteins, draws maximal attention to affinity as a key parameter affecting the efficacy of interacting ligands. Furthermore, for most receptors, the response to a given drug primarily depends on the competition between this exogenous orthosteric ligand and the endogenous agonist. In this respect, the control implemented by drugs acting in a competitive manner should always be seen as non-permissive [47]. For example, when several orthosteric ligands are present at identical concentrations, the one showing the highest affinity for the receptor will dictate a defined response spectrum, ruling out alternative control by other ligands with lower affinity. This situation contrasts with the flexibility offered by allosteric ligands acting at an auxiliary site of the receptor in a non-competitive manner. Indeed, binding of an allosteric ligand is thought to impose structural changes in the receptor conformations, influencing both recognition by orthosteric ligands and coupling to G proteins. In addition, by affecting the constitutive activity, allosteric ligands could implement direct control on the receptor signaling and thus show proper efficacy [51].

Under the concept of non-competitive antagonism, drugs acting at allosteric sites of GPCRs should not be regarded as strict positive or negative modulators of G-protein activation by orthosteric ligands. As a result, allosteric modulators offer the possibility to achieve fine-tuning of the pharmacological responses. Indeed, several recent examples indicate that the nature and extent of this modulation differ with respect to the cellular or tissue environment and to the orthosteric ligand examined (probe dependency). For example, regional differences in the rat brain have been observed during the positive allosteric modulation of adenosine A_1 receptors by compound T62 [52]. Avoiding widespread activation of adenosine transmission, this tissue specificity supports the advantage of T62 over conventional agonists in the treatment of neuropathic pain and might be explained by discreet differences in endogenous levels of adenosine or interacting G proteins. In addition, a convincing example

Box 1. From intrinsic efficacy to functional selectivity

The complexity of the responses associated with several drugs that act on GPCRs is best explained by considering a model in which the receptor can adopt multiple conformations (Figure Ia). These conformations (R, R′ and R″) differ in their functional coupling with G proteins. In the absence of ligand, an equilibrium spontaneously establishes between the conformations, supporting the existence of distinct constitutive activities of unrelated G proteins. The pharmacodynamic properties of orthosteric drug (A and B) simply reflect the affinity of these drugs for selected conformations, which are stabilized at the expenses of others. Thus, these drugs tend to modify the equilibrium, promoting the activation of some G proteins while reducing that of others. In the example shown, drug A shows a high affinity for conformation R′ and a modest affinity for R″, whereas drug B shows a high affinity for conformation R″ and an affinity for R′ so low that this conformation is destabilized. As a consequence, drug A behaves as an agonist when considering coupling to either G1 or G2, although with different intrinsic efficacies. By contrast, drug B behaves as a high-efficacy agonist when considering coupling to G2 but as an inverse agonist when examining the response involving G1. Notably, when both ligands are present, B will be viewed as an agonist when measuring G2 activation, but as an antagonist when measuring the G1-dependent response elicited by A.

Both the spontaneous equilibrium (dictating the receptor constitutive activity) and the pharmacodynamic properties of orthosteric ligands (A) are likely to be influenced by intrinsic (e.g. receptor mutations glycosylation, phosphorylation and dimerization) and extrinsic (e.g. interacting proteins, signaling partners and allosteric ligands) factors, which accounts for the differences in constitutive activities and functional responses to drugs observed in distinct tissues or cell types (Figure Ib). This model explains how alterations in constitutive activity can switch the properties of a ligand from those of partial agonist to those of an inverse agonist (termed a ‘protean agonist’; see the activity of drug A on G2 activation).

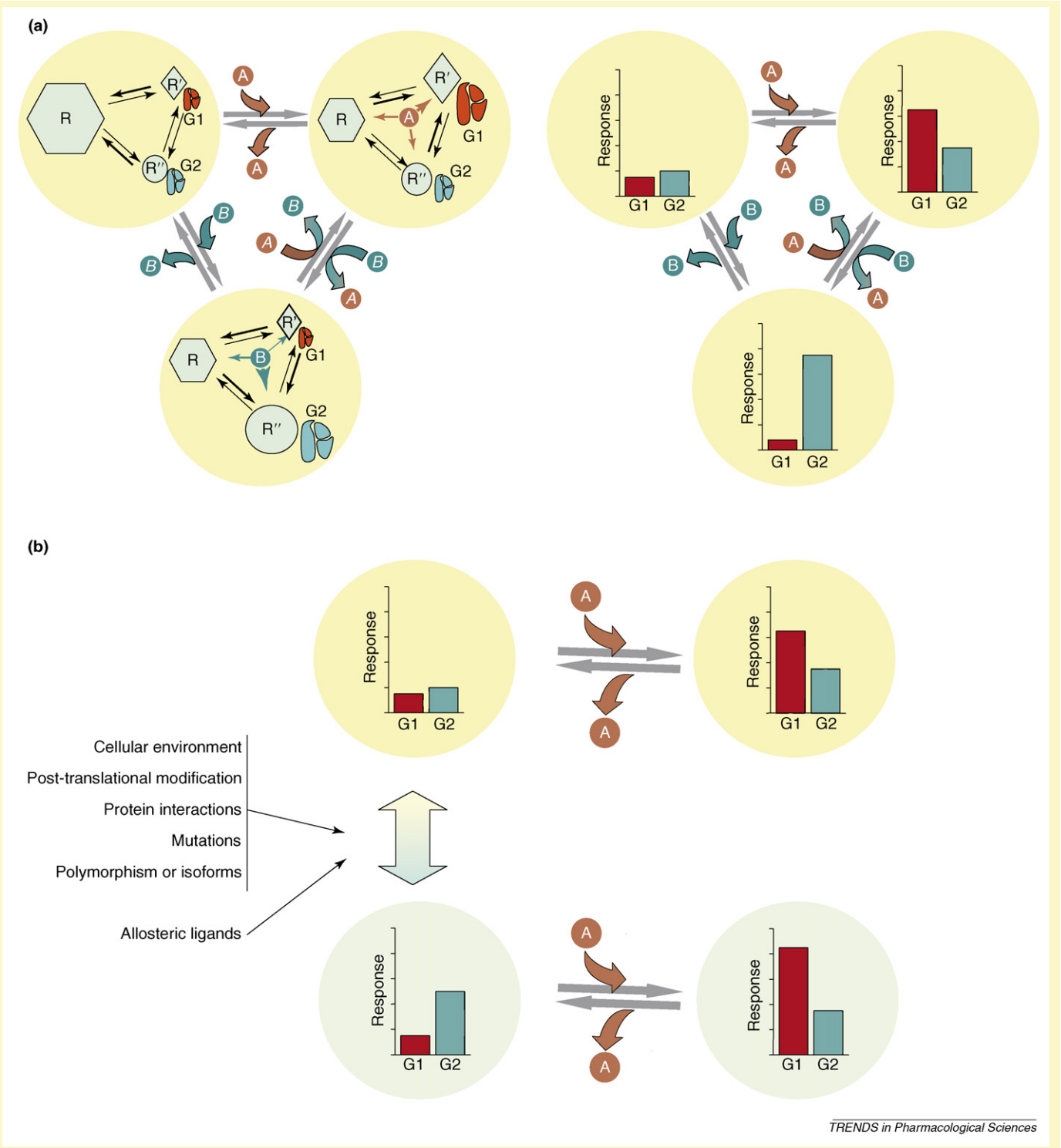

Figure I. The multiple conformations model. **(a)** An example of the complex equilibrium between one inactive (R) and two active (R′ and R″) conformations of the receptor, and the influence of two ligands (A and B) showing distinct functional properties. **(b)** An illustration of the influence of extrinsic and intrinsic factors on the constitutive activity of a given GPCR and on the functional responses to a ligand (A).

of the reciprocal influence of an allosteric ligand on the activity of distinct orthosteric probes is provided by Org-27569 and related analogues that interact with CB_1 cannabinoid receptors. These ligands increase the affinity of the agonist CP 55 940, while decreasing the affinity of the inverse agonist SR 141716A [53]. Indeed, cannabinoid receptors constitute a good example of GPCRs with a diversity of intracellular signals that can be manipulated with various ligands acting at orthosteric or allosteric sites (Box 2).

Because the activity of allosteric modulators is best explained by alterations in receptor conformations, it might be predicted that such ligands could also influence the functional selectivity of orthosteric ligands. Indeed, Maillet *et al.* [54] recently reported on the properties of LPI805, identified as a potent modulator of the NK2

Box 2. Versatility of drugs and endogenous agonists acting at cannabinoid receptors

Δ^9-Tetrahydrocannabinol, the main psychoactive constituent of the plant *Cannabis sativa* acts as an agonist at the cannabinoid CB_1 and CB_2 receptors. The CB_1 receptor is present in the nervous system and is involved in the modulation of neurotransmission, whereas CB_2 seems to be mainly associated with the immune system. Both receptors recognize endocannabinoids (anandamide and 2-arachidonylglycerol) with similar affinity and inhibit adenylyl cyclase through $G_{i/o}$ proteins that are sensitive to pertussis toxin. In addition to natural and synthetic non-selective agonists such as Δ^9-tetrahydrocannabinol, anandamide, HU210 and CP 55 940, which have different intrinsic activities, several CB_1- or CB_2-selective antagonists have been developed (Figure I), offering promising therapeutic perspectives [58]. As frequently observed, most of these antagonists have turned to be inverse agonists with variable intrinsic efficacy. Lastly, related to its low efficacy, the partial agonist AM1241 has been shown to behave as a protean agonist in cells expressing recombinant CB_2 receptor [23].

Extensive characterization of the intracellular signaling triggered on activation of the CB_1 and CB_2 receptors has revealed their coupling to multiple G proteins in transfected cells, in the rat brain and in cell lines that constitutively express these receptors. Thus, the CB_1 receptor interacts with several subtypes of G_i protein, G_0, G_s and $G_{q/11}$ [59–61], whereas the multiplicity of CB_2 receptor coupling seems to be restricted to distinct G_i proteins [62]. Careful examination of the pharmacodynamic properties of the conventional agonists of these receptors, including endocannabinoids, has revealed that all of them show functional selectivity [62–65]. Thus, CB receptor ligands from unrelated chemical families have been found to differentially promote coupling with different G proteins. Considering the pleiotropic functions of the cannabinoid systems, the development of ligands endowed with functional selectivity would certainly contribute to their putative clinical efficacy.

Notably, presence of the dopamine D2 receptor in the same cells has been shown to influence functional coupling of the CB_1 receptor with distinct G proteins, suggesting that the well-documented cross-talk between the dopamine and cannabinoid systems might occur at the level of the specificity of G-protein coupling [66]. Indeed, a key issue in cannabinoid pharmacology lies in dissociating the putative benefit of these drugs in the treatment of pain or psychiatric disorders from their likelihood to induce addiction. Of particular interest is the recent observation that, in a model of neuroblastoma cells expressing the CB_1 receptor, HU 210 and CP 55 940 increased expression of tyrosine hydroxylase, whereas unrelated agonists of the aminoalkylindole family such as WIN55 212–2 reduced expression of this enzyme involved in catecholamine synthesis [67]. Although the physiological relevance of this observation remains mostly speculative, it could be argued that the positive or negative control of cannabinoid agonists on dopamine transmission might affect the modulation of reward circuits in the basal forebrain.

Lastly, a recent study has uncovered the possibility of modulating the response to CB_1 receptor agonists with compounds that interact at an allosteric site [53]. Indeed, drugs that could influence either the affinity of the orthosteric ligands or their intrinsic efficacy at CB receptors constitute potential alternatives or adjuncts to conventional agonists and antagonists for selective manipulation of the cannabinoid systems.

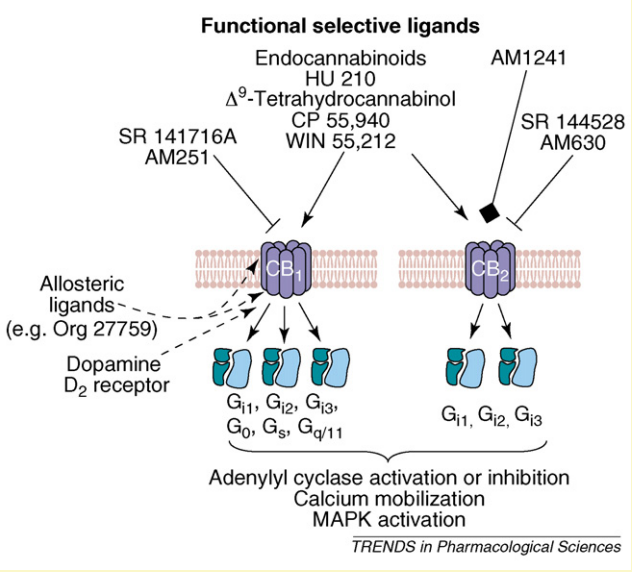

Figure I. Cannabinoid receptors: diversity of ligands and associated intracellular signals.

neurokinin receptor that couples to both G_q and G_s proteins in response to the endogenous peptide neurokinin A. In cells expressing the recombinant NK2 receptor, LPI805 was found to impair the neurokinin-A-mediated increase in cyclic AMP production while enhancing activation of phospholipase-C-dependent intracellular Ca^{2+} mobilization [54]. These observations suggest that LPI805 implements permissive control on the NK2 receptor because it stabilizes G_q coupling while destabilizing G_s coupling. Thus, the permissive properties of allosteric modulators extend beyond the control of ligand binding to the orthosteric site because their selective interference might also operate at the level of G-protein activation (Box 1).

Although only a few examples of the clinical use of allosteric modulators of GPCRs have been proposed so far [55], the design and characterization of such ligands have recently received considerable attention [56]. Therefore, there is no doubt that their use in the treatment of diverse human pathologies will rapidly be proposed either in monotherapy or in combination with conventional drugs. Indeed, used alone these drugs offer the potential to increase the efficiency of endogenous transmission without the risk of inappropriate stimulation of the whole system, as has been recently shown for drugs acting on the type 5 metabotropic glutamate receptor [57]. Alternatively, their combination with orthosteric ligands could help to gain pharmacological discrimination among closely related receptor subtypes.

Concluding remarks

Experimental data acquired in the 1980s and 1990s revealed the unexpected complexity of cell signals associated with GPCR activation. More recent studies have demonstrated the possibility of independently manipulating these signaling pathways by using appropriate ligands. Indeed, several new ligands with multifaceted pharmacological profiles have been developed. In addition, singular, previously undocumented properties of well-established drugs have been highlighted. The new concepts related to the complex influence of ligands on responding systems call for a revision of the definition of agonism and intrinsic efficacy. Indeed, as the revised theoretical models help to predict the behavior of diverse types of ligand, so this complexity should be regarded as an opportunity to gain pharmacological specificity and to increase therapeutic efficacy.

To exploit this potential, however, it is essential to characterize and understand further the importance of multifaceted GPCR activity in the physiology of cell communication. In this respect, all of the newly described ligands with distinct functional properties not only represent putative future drugs, but also constitute relevant tools for exploring GPCR function in physiological and pathological processes.

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