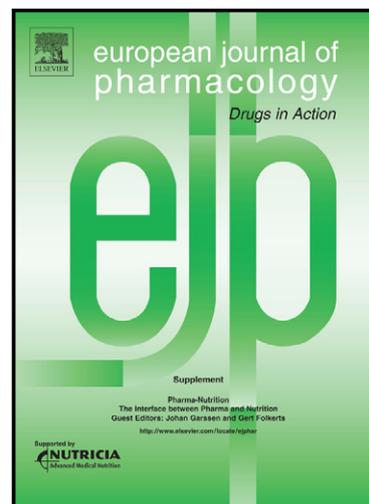


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Antihistaminergics and inverse agonism. Potential therapeutic applications.**Federico Monczor^{1,4}, Natalia Fernandez^{1,4}, Carlos P. Fitzsimons², Carina Shayo^{3,4} and Carlos Davio^{1,4}.**

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Running head: G-protein sequestering by GPCR inverse agonists.

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Abstract

The accurate characterization of the molecular mechanisms involved in the action of receptor ligands is important for their appropriate therapeutic use and safety.

It is well established that ligands acting at the histamine system currently used in the clinic exert their actions by specifically antagonizing G-protein coupled H₁ and H₂ receptors. However, most of these ligands, assumed to be neutral antagonists, behave as inverse agonists displaying negative efficacy in experimental systems. This suggests that their therapeutic actions may involve not only receptor blockade, but also the decrease of spontaneous receptor activity.

The mechanisms whereby inverse agonists achieve negative efficacy are diverse. Theoretical models predict at least three possible mechanisms, all of which are supported by experimental observations. Depending on the mechanism of action engaged, the inverse agonist could interfere specifically with signaling events triggered by unrelated receptors. This possibility opens up new venues to explain the therapeutic actions of inverse agonists of the histamine receptor and perhaps new therapeutic applications.

Keywords: Histamine receptors, antihistaminergics, inverse agonism, adverse effects.

1. Histamine receptors and its role in human health.

Histamine is a biogenic amine synthesized from L-histidine by histidine decarboxylase. This amine plays an important role in human health and disease. It exerts its biologic effects acting through four receptor subtypes, termed H₁, H₂, H₃, and H₄. These receptors differ in their expression, molecular characteristics, resulting in differences in associated signal transduction pathways and function (Hill et al., 1997; Leurs et al., 2009). Nevertheless, all types of histamine receptors belong to the seven transmembrane spanning family of receptors, also known as GPCRs.

The histamine H₁ receptor is in fact the classical histamine receptor. This is one of many receptors that influence cellular homeostasis by modifying phospholipase C activity via interactions with Gproteins from the Gαq/11 family. Following the original observations of Hokin and Hokin in 1953 (Hokin and Hokin, 1953), histamine-induced incorporation of ³²P in phosphatidylinositol was first reported in 1975 in the rat brain *in vivo* (Friedel and Schanberg, 1975). In a seminal study in the same system, ligand binding with specific H₁ receptor ligands paralleled the development of histamine-induced incorporation of ³²P into phosphoinositides, demonstrating for the first time histamine-induced activation of phospholipase C (Subramanian et al., 1981). Currently, H₁ receptor-induced phosphoinositide responses mediated by Gαq/11 activation have been described in a variety of central and peripheral tissues and in isolated cell systems as well (Smit et al., 1999).

The observation that histamine-evoked gastric acid secretion could not be blocked with classical

antihistamines (H_1 antagonists), led to the conclusion that other histamine receptors were involved. The receptor involved in mediating histamine-dependent gastric acid secretion was subsequently termed histamine H_2 receptor (Black et al., 1972). It is generally accepted that H_2 receptor is coupled to adenylyl cyclase through Gas. However, it has been reported that the H_2 receptor can also activate phospholipase C (Davio et al., 1995; Davio et al., 2002) and perhaps both pathways simultaneously (Delvalle et al., 1992; Wellner-Kienitz et al., 2003). In fact, the activation of signal transduction pathways is highly dependent on the cellular system under study and outcome of H_2 receptor activation is probably mediated by various intracellular mediators.

A third histamine receptor, H_3 , was originally postulated in 1983 as a putative histamine autoreceptor responsible for controlling histamine release in rat cerebral cortical slices (Arrang et al., 1988). Since then, the H_3 receptor has been shown to be coupled to modulation of adenylyl cyclase activity via Gai/0 activation, and has been associated to inhibition of neurotransmission in central (Clapham and Kilpatrick, 1992; Schlicker et al., 1993; van der Werf and Timmerman, 1989) and peripheral tissues (Burgaud and Oudart, 1993; Imamura et al., 1995; Ishikawa and Sperelakis, 1987).

More recently, a fourth histamine receptor has been described. The H_4 receptor is also a GPCR and it is coupled to Gai/0, resulting in modulation of various signal transduction pathways (Oda et al., 2000). The H_4 receptor is prominently expressed in medullary and peripheral hematopoietic cells, namely eosinophils, neutrophils and $CD4^+$ T cells (Zhu et al., 2001). The H_4 receptor is involved in histamine-induced increases in intracellular calcium in human eosinophils (Raible et al., 1994), facilitating terminal myeloblast and promonocyte differentiation (Nakaya and Tasaka, 1988).

Until now, the most clinically relevant uses of histamine receptor ligands are achieved through the regulation of H_1 or H_2 receptors, which are widely expressed in most tissues (Bakker et al., 2002). In this regard, H_1 antihistamines are used in the treatment of several allergic conditions, such as rhinoconjunctivitis, urticaria, and atopic dermatitis. Traditionally, the efficacy of H_1 antihistamines in allergic disorders was attributed primarily to inhibition of H_1 receptors in endothelial cells of the postcapillary venules, resulting in decreased vascular permeability, exudation of fluid, protein and cells and increased peripheral resistance (Leurs et al., 2002). Nevertheless, H_1 antagonists also inhibit the effect of histamine at H_1 receptors in the airway smooth muscle and on afferent C fibers, causing bronchodilation and decreased mucosal and cutaneous itch, respectively.

H_2 antagonists have proved to be very active agents for the treatment of duodenal and gastric ulcers, reflux, esophagitis and the Zollinger-Ellison syndrome. Since H_2 receptors are abundant throughout the body, it is rather remarkable that H_2 antagonists have not found other applications. Several studies have claimed beneficial effects of H_2 antagonists in, e.g. viral diseases and alleviation of psoriasis (Nielsen, 1991), with no conclusive results.

The identification of H_3 and H_4 receptors and their pattern of expression has led to a renewed interest in the potential homeostatic role of histamine in the brain, besides their established pleiotropic regulatory functions in the periphery. Although no central nervous system disease has been associated directly to histamine dysfunction until now, the H_3 receptor is recognized as a drug target for neuropathic pain, sleep-wake disorders, including narcolepsy, and cognitive impairment associated with attention deficit hyperactivity disorder, schizophrenia, Alzheimer's, or Parkinson's disease and the first specific H_3 receptor ligands have already entered phase I–III clinical trials (Tiligada et al., 2011). However, before their introduction in the market, H_3R ligands may require further testing to identify the determinants of their clinical efficacy.

Nowadays, more than forty H_1 antihistamines are therapeutically available worldwide. However, some of them, such as astemizole and terfenadine, have been associated with cardiac toxic effects

and are no longer approved for use in most countries (Yap and Camm, 2002). On the other hand, H₂ antagonists, widely used as antiacids, were overcome by proton-pump inhibitors, due to their more favorable efficacy and safety profiles. However, a recent survey revealed that between the twenty most commonly used prescription and over-the-counter drugs are antiallergic formulations acting at H₁ (difenhydramine and loratadine), and antiacids acting at H₂ receptors (ranitidine) (Kaufman et al., 2002).

From the mechanistical point of view, it has been proven *in vitro* that clinically relevant histamine receptor ligands do not act as simple antagonists, but rather as inverse agonists stabilizing inactive forms of the H₁ and H₂ receptors (Leurs et al., 2002; Smit et al., 1998). These observations have led to the pharmacological reclassification of histamine H₁ and H₂ specific antagonists. The concept of inverse agonism arose from experimental observations showing that certain drugs were able to reduce the activity of receptor systems that were active in the absence of agonists. The inverse agonists possess, in consequence, negative efficacy. According to existing receptor occupancy models, there are several mechanisms whereby inverse agonists exert their negative efficacy, and each one may lead to distinct consequences. However, whether inverse agonism is essential or important for these drugs to exert their medicinal actions has not been clarified yet. This point deserves attention due to the widespread use of histamine receptor ligands reclassified as inverse agonists in clinical treatments. In the following sections we will discuss these aspects of clinically used histamine receptor ligands in the context of their pharmacological safety.

2. Spontaneous receptor activity. The rise of the inverse agonism concept.

Due to the recognition that many receptors are able to spontaneously activate downstream effector protein (reference) pharmacology experienced a rebirth in the understanding of the molecular mechanisms triggered by molecules that modulate receptor function. As a result, there was a significant realization of the importance of explicitly formulating spontaneous receptor activity observed both in natural and artificial systems (Costa and Cotecchia, 2005; Kenakin, 1995). This realization led to the formulation of the two-state model (TSM) (Leff, 1995; Robertson et al., 1994) and related reaction schemes developed later (Bindsvlev, 2004; Hall, 2000; Weiss et al., 1996a; Weiss et al., 1996b; Weiss et al., 1996c).

Increasing numbers of compounds previously classified as antagonists were shown to inhibit this spontaneous or constitutive receptor activity (Kenakin, 2004). These thought-to-be antagonists with negative efficacy are now known as 'inverse agonists', whereas compounds that antagonize the inhibitory effect of agonists and inverse agonists without having an effect of their own are still termed antagonists, or more precisely neutral antagonists (Chidiac, 2002; Kenakin, 2004; Milligan et al., 1995; Strange, 2002).

Classically, efficacy (whether positive, negative or zero) was thought as a separate property unrelated to affinity. However, in thermodynamic terms, this representation presents a paradox because the molecular forces that control affinity are the same as those controlling efficacy (Kenakin and Onaran, 2002). Taking this into account, it is not surprising that 85% of the ligands formerly known as antagonists have been shown to possess negative efficacy (Kenakin, 2004).

3. Theoretical models of ligand-receptor occupancy and the mechanisms of inverse agonism.

Receptor activity measured in functional studies can be either basal (i.e. spontaneous, constitutive and ligand-independent), or agonist-induced. This experimental observation is independent of the model used to interpret the system. However, different spontaneous receptor conformations can be hypothesized to be responsible for the basal activity, comprising a "receptor native ensemble". Hence, as we are going to discuss in the next paragraph, the uniformity of the concept of constitutive receptor activity is apparently challenged when the spontaneous receptor species (and

therefore the source of the receptor basal activity) are explicitly formulated (Kenakin, 2002; Onaran and Costa, 1997) (Fig. 1).

For the TSM, receptors can spontaneously adopt only two forms, the resting or inactive state (R), and the active one (R*), to which the basal activity of the system is formally attributed. However, when accessory proteins (G-proteins) are included in the models, as in the extended ternary complex model (ETCM) (Samama et al., 1993), the native ensemble involves three distinct receptor forms including the inactive (R) and the active species (R*), but also an active G-protein coupled receptor form that is considered responsible for basal activity (R*G).

Similarly, the cubic ternary complex model (CTCM) (Weiss et al., 1996a; Weiss et al., 1996b; Weiss et al., 1996c) adds one more receptor form to the native ensemble, allowing receptor to couple to G-protein in an inactive form (RG).

The CTCM was originally proposed in an attempt to explore the mathematical and pharmacological implications that can be derived from permitting G-proteins to interact with receptors in their inactive and active forms irrespectively. However, it is worth noting that regardless of its theoretical origins, there are empirical observations supporting the CTCM. Some mutants of α_{1B} -adrenergic receptors are able to spontaneously couple to G-protein inactivating it (Chen et al., 2000), and this has also been observed for non-mutant serotonin 5HT₇ and histamine H₂ receptors (Andressen et al., 2006; Tubio et al., 2010).

The three models and their relations are schematically shown in Fig. 2. The fact that these models assume differences in the receptor states that exist spontaneously, implies these models could be used to explain ligand-dependent selection and stabilization of different preexisting receptor conformations. According to the law of mass action, when a factor affects previously established equilibriums, the receptor species are redistributed to re-establish equilibrium. Consequently, for all the models, inverse agonists exert their effect favoring the inactive receptor species at the expense of the active ones. However, the particular receptor species favored changes depending on the model. In this way, according to the TSM, inverse agonists suppress the spontaneous activity of the receptors by stabilizing them in an inactive state. Similarly, in the ETC model, inverse agonists may act by preferentially binding to the R state over the R* state ($\alpha < 1$). In both cases, R*G species will be depleted as more receptor transforms into ligand-bound inactive R (LR), resulting in a decreased constitutive activity. There are several experiments that confirm this model, at least for the α_2 - and β_2 -adrenergic receptors (Samama et al., 1993; Wade et al., 2001). Additionally, for the ETCM, ligands could bind to uncoupled states of the receptor (R and R*) in preference to the coupled state (R*G) ($\gamma < 1$). This feature was described for 5-HT_{1A} and 5-HT_{2C} receptors, cardiac muscarinic receptors (M₂ receptors), and dopamine D₂ receptors (De Lean et al., 1982; Martin et al., 1984; McLoughlin and Strange, 2000; Westphal and Sanders-Bush, 1994).

Another possibility, only accounted for in the CTCM, is that inverse agonists bias the receptor to a G-protein coupled but inactive conformation of the receptor (RG). It is worth noting that in the ETC model a ligand with high affinity for receptor species coupled to G-proteins would necessarily elicit a response. In contrast, the CTC model allows a ligand with high affinity for a receptor species coupled to G-protein to behave as an inverse agonist.

For the ETC model a ligand that facilitates receptor activation ($\alpha > 1$), promotes that the active receptor has more affinity for G-protein than the inactive form ($\beta > 1$), and improves G-protein coupling ($\gamma > 1$), is defined as an agonist. However, a negative cooperativism among those receptor modifications may exist in the sense that ligands might behave as inverse agonists. This point is a distinctive feature of the CTC model and is made possible by proposing the δ parameter, which represents the synergism among the receptor modifications (activation, binding to ligand, or coupling to G-protein). Therefore in the conceptual frame of the CTC it can be theoretically

predicted, and then empirically proved, that an inverse agonist can exert its effect by stabilizing a G-protein coupled but inactive form of the receptor. Consequently, it can be inferred that if the G-protein is in limiting quantities, the ligand will be able to interfere with the signaling of other unrelated GPCRs that share the same signaling cascade. This effect can be interpreted in terms of a G-protein “molecular kidnapping“, mediated by the inverse agonist-bound receptor (Fig. 3). This theory explains some experimental observations made not only for ligands acting at H₁ and H₂ histamine receptors (Fitzsimons et al., 2004; Monczor et al., 2003), but also for ligands acting at μ -opioid and CB₁ cannabinoid receptors, that interfere with the signaling of other related receptors (Bouaboula et al., 1997; Brown and Pasternak, 1998). This view can also take account of some striking results obtained for D₂ dopamine and M₃ muscarinic receptor ligands, showing that an inverse agonist can exert its effects without promoting the expected receptor G-protein uncoupling (Dowling et al., 2006; Wilson et al., 2001).

This interference of an inverse agonist of a given GPCR on the signaling of a non related receptor highlights the importance of the study of the mechanistic basis of action of ligands with negative efficacy. Taking into account that this may be a generalized feature of several ligands with clinical uses, a deeper understanding of this phenomenon could help to rationalize otherwise unexpected drug effects. Regarding this, the current as well as potential uses of inverse agonists of the histaminergic receptors in clinical treatment are discussed in the next section.

4. Potential clinical uses of inverse agonists of the histaminergic receptors.

When inverse agonism was first described, there were concerns on whether it was of therapeutic relevance *in vivo* or just a curious observation induced by laboratory conditions (Milligan et al., 1995). Since then, hundreds of well-known drugs of extensive clinical use have been tested and reclassified for this property. However, questions on the therapeutic relevance of inverse agonism remain.

Prominent examples of extensively used drugs formerly reclassified as antagonists and currently reclassified as inverse agonist due to their negative efficacy *in vitro* include: propranolol, alprenolol, pindolol, and timolol used for treating hypertension, angina pectoris, and arrhythmia acting on the β_2 -AR; metoprolol and bisoprolol used for treating hypertension, coronary heart disease, and arrhythmias acting on β_1 -AR; cetirizine and loratadine for treating allergies and hay fever acting on histamine H₁ receptor; cimetidine and ranitidine used to treat heartburn and peptic ulcer acting on the histamine H₂ receptor; prazosin and phentolamine for treating hypertension acting on α_1 -adrenergic receptors; atropine used as premedication for anesthesia and pirenzepine used for treatment of peptic ulcer acting on the muscarinic receptors and naloxone used for treating heroin overdose acting on μ -opioid receptor (Kenakin, 2004).

At a first glance, it may seem there is no theoretical or practical reasons to believe that inverse agonists should have an intrinsic benefit over an antagonist in many situations where drugs are clinically used. In general, drugs are simply used to regulate the release of an endogenous agonist or to compete with it and limit its actions. Therefore potency, bioavailability and selectivity seem the key issues to consider. However, in a range of circumstances, specific benefits of using an inverse agonist can be envisaged.

A first requirement to conceive an advantage in using an inverse agonist over an antagonist is that the receptor displays constitutive activity *in vivo*. In this sense, the constitutive activity of GPCRs in native *in vivo* systems has been proved only for few receptors, specifically including H₂ and H₃ histamine receptors, acting at CNS. The H₂ receptor was shown to display spontaneous activity in the *substantia nigra* region and this activity regulated serotonin independently of the local histamine tone. This observation has implications not only for Parkinson's disease, where histamine levels in the *substantia nigra* are increased, but also for other neuropsychiatric disorders in which serotonin is pivotal (Threlfell et al., 2008). In turn, constitutive activity of native H₃ receptors was shown in

rodent brain controlling histamine-dependent neuronal activity *in vivo*. The activation of histaminergic neurons, which promotes arousal and attention and improves learning in normal animals, has been proposed as a symptomatic therapeutic approach in human attentional and ageing disorders, and such an effect is more likely to be obtained with H₃ receptor inverse agonists rather than with neutral antagonists (Morisset et al., 2000).

On the other hand, there are clinical instances where the pathological entity is a constitutively active GPCR, which produces physiological response in the absence of endogenous agonists. In these circumstances, the underlying condition may be only effectively treated with inverse agonists. Mutations, which may be preserved in the germ line, have been shown to occur in GPCRs and result in constitutive receptor activity in patients with clinical syndromes, such as Jansen's metaphyseal chondrodysplasia or congenital hyperthyroidism (Bastepe et al., 2004; Davies et al., 2005; Seifert and Wenzel-Seifert, 2002). Most of these diseases are associated with the endocrine system, although visual alterations due to rhodopsin mutations (Dryja et al., 1993; Keen et al., 1991) and some viral infections were also related to constitutively active forms of GPCRs (Arvanitakis et al., 1997; Fitzsimons et al., 2006; Maussang et al., 2009).

There are also many examples where inverse agonists may be useful for cancer treatment. For instance, high levels of specific GPCR expression have been described in tumour cells, where it has been shown that their endogenous ligands, present at high levels in the tumour cells (el Battari et al., 1988; Gespach et al., 1988; Korman et al., 1986; Virgolini et al., 1994), enhance cell proliferation (Kroog et al., 1995; Pincus et al., 1990; Zurier et al., 1988). The histamine H₂ receptor represents one interesting example of this property (Davio et al., 1995; Fitzsimons et al., 1999; Molnar et al., 2001). Interestingly, some early studies made in rats bearing experimental mammary adenocarcinomas showed that anti H₂ ligand administration resulted in the remission of a significant number of tumours, while also increased the survival of treated animals (Cricco et al., 1993; Rivera et al., 1993). Moreover, diverse clinical reports suggest that H₂ antagonists/inverse agonists have potential beneficial effects in the treatment of advanced malignant diseases such as colorectal cancer, gastric cancer, liver metastasis, multiple myeloma, chronic lymphocytic leukaemia and melanoma (Burtin et al., 1988; Nielsen, 1996; Nielsen and Kikuchi, 1993). In those cases, inverse agonists would block not only the effects of humoral activation in cancer cells (i.e. secreted histamine) but also increased basal activity of these receptors in the tumour due to receptor overexpression.

The studies summarized above provide supporting evidences for the relevance of pathological constitutive activity, suggesting the potential therapeutic values of its modulation. However, these potential advantages need to be tested for correlation with clinically relevant beneficial or detrimental effects. For this reason, and two decades after the discovery of inverse agonism on GPCRs, we have just begun exploring evidence of the clinical relevance of inverse agonists. However, it is worth noting that none of the above described diseases that involve pathological consequences of a constitutively active GPCR are in fact treated with inverse agonists. As suggested by Kenakin, translational research of inverse agonism will show whether new therapeutic options will emerge (Kenakin, 2004), especially for silencing the constitutively active mutant receptors that cause the diseases described above.

Up to this point, we have described only examples of desirable acute effects of inverse agonists. Alternatively, inverse agonists may require chronic administration and therefore exert some undesirable effects. For instance, receptor up-regulation could occur as it was observed for the H₂ receptor after long-term treatment with cimetidine or ranitidine (Osawa et al., 2005; Smit et al., 1996), providing a plausible explanation for the tolerance to these inverse agonists after prolonged clinical use. In the same way, Milligan and colleagues showed that inverse agonists also up-regulate β_2 - and α_{1B} -ARs (Georgieva et al., 2008; Lee et al., 1997; MacEwan and Milligan, 1996).

Recently, it has been shown that the CB₁ receptor inverse agonist rimonabant suppresses receptor constitutive activity in the ventral tegmental area and basolateral amygdala, reducing weight gain and food intake, but also causing anxiety and reduced motivation for reward, while NESS0327, acting as a neutral antagonist on the same receptor, is equally effective on reducing weight gain and food intake, but lacks the negative effects associated with rimonabant's inverse agonism. Considering that rimonabant has been discontinued for human treatment because its use was occasionally associated with negative effects and suicidality, the findings suggest that neutral CB₁ receptor antagonists can treat obesity more efficiently and safely than inverse agonists (Meye et al., 2012).

In conclusion, in this review we not only emphasize the importance of proper classification of ligands of constitutively active receptors, but also the importance of elucidating the molecular mechanisms by which these ligands exert their actions in terms of their clinical applications and pharmacological effects. An inverse agonist may act by three different ways: biasing the system to an inactive form of the receptor; facilitating the G-protein uncoupled forms; or promoting a G-protein coupled but inactive form of the receptor. If the preferred mechanism for a given ligand would be the third one, it may not only diminish the activity of the specific receptor but it may also interfere with the signalling of other non-related GPCRs. This is the case for some cannabinoid CB₁ (Bouaboula et al., 1997; Georgieva et al., 2008), μ opioid (Brown and Pasternak, 1998) and dopamine D₂ receptor (Wilson et al., 2001) ligands and, of central interest for this review, also for H₁ and H₂ ligands (Fitzsimons et al., 2004; Monczor et al., 2003).

This interference with the signaling of other GPCRs, may bring some unexpected effects that would not be observed with inverse agonists that do not promote G-protein sequestration, and could rationalize the appearance of pharmacological interactions and/or side effects that would be otherwise difficult to explain.

Ligands acting at histaminergic system are among the most widely prescribed and over-the-counter-sold drugs in the world, and there is a trend to use them as long-term therapeutics rather than restricting them to the treatment of short-term manifestations. Within this context, progress in the understanding of their mechanism of action is of crucial importance to improve their safety and specificity. Taking into account the clinically widespread use of histamine antagonists acting at H₁ and H₂ receptors in the treatment of several human diseases, the proper pharmacological classification of these ligands and the accurate characterization of their mechanism(s) of action is of great importance.

Legends for figures

Figure 1.

The figure shows the theoretical receptor species available for ligand binding. Inactive receptor conformations are presented on the left and the active ones on the right of the equilibrium arrows. Depending on the specific ligand-receptor interactions the ligand can redistribute the spontaneous receptor conformations, thereby favoring or disfavoring basal activity. This action of the ligand results from binding preferentially inactive or active species, or both, depending on whether the drug is an agonist, an inverse agonist, or eventually behaves as a neutral antagonist not affecting the spontaneous receptor activity.

Figure 2.

The figure shows the three most common models used to describe GPCR behaviors. The models describe inactive receptor species (R) in spontaneous equilibrium with an active state (R*) responsible for the basal activity of the system. In the extended ternary complex model (center), activation of the receptor could be followed by binding of R* to the G-protein (G). The cubic ternary complex model (right) implies the same assumption, but also allows the ligand-bound or unbound inactive-state receptor species (LR or R) to form a non-signaling complex with the G-protein (LRG or RG, respectively). Dotted arrows point to species that could be favored by inverse agonists, diminishing receptor basal activity. It should be noted that there is a trend in the complexity where following scheme contains the previous, and that the liganded inactive receptor species are different for each model, implying different mechanisms of action accounting for inverse agonism. These different mechanisms are discussed in the main text.

Figure 3.

The figure represents how an inverse agonist, acting on a specific receptor species and promoting the coupling to the G-protein to a receptor inactive state (LRG complex, see figure 2) can interfere with the signaling of a second unrelated receptor that transduces its signal through the same G-protein. The potential implications of such interference are discussed in the main text.

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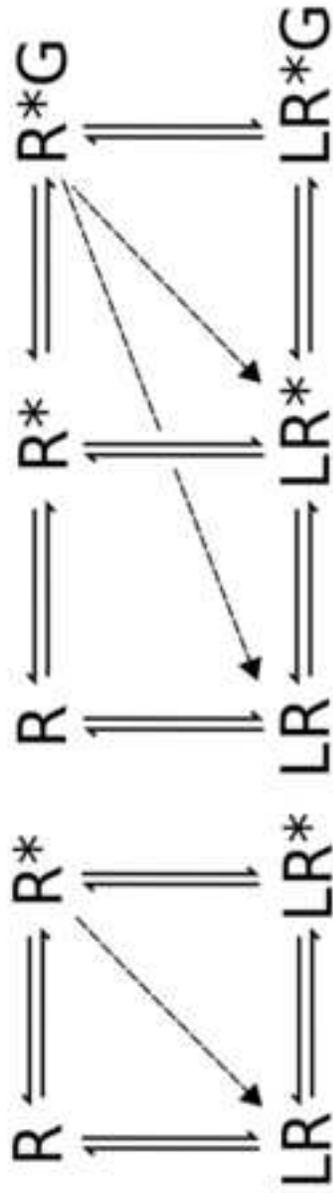
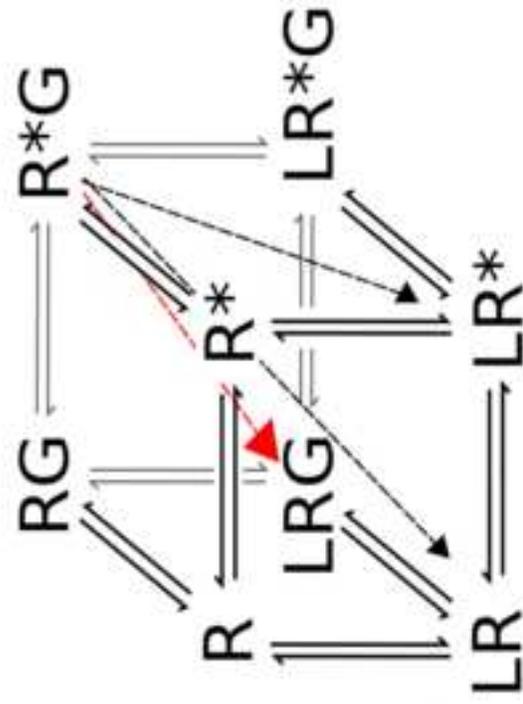
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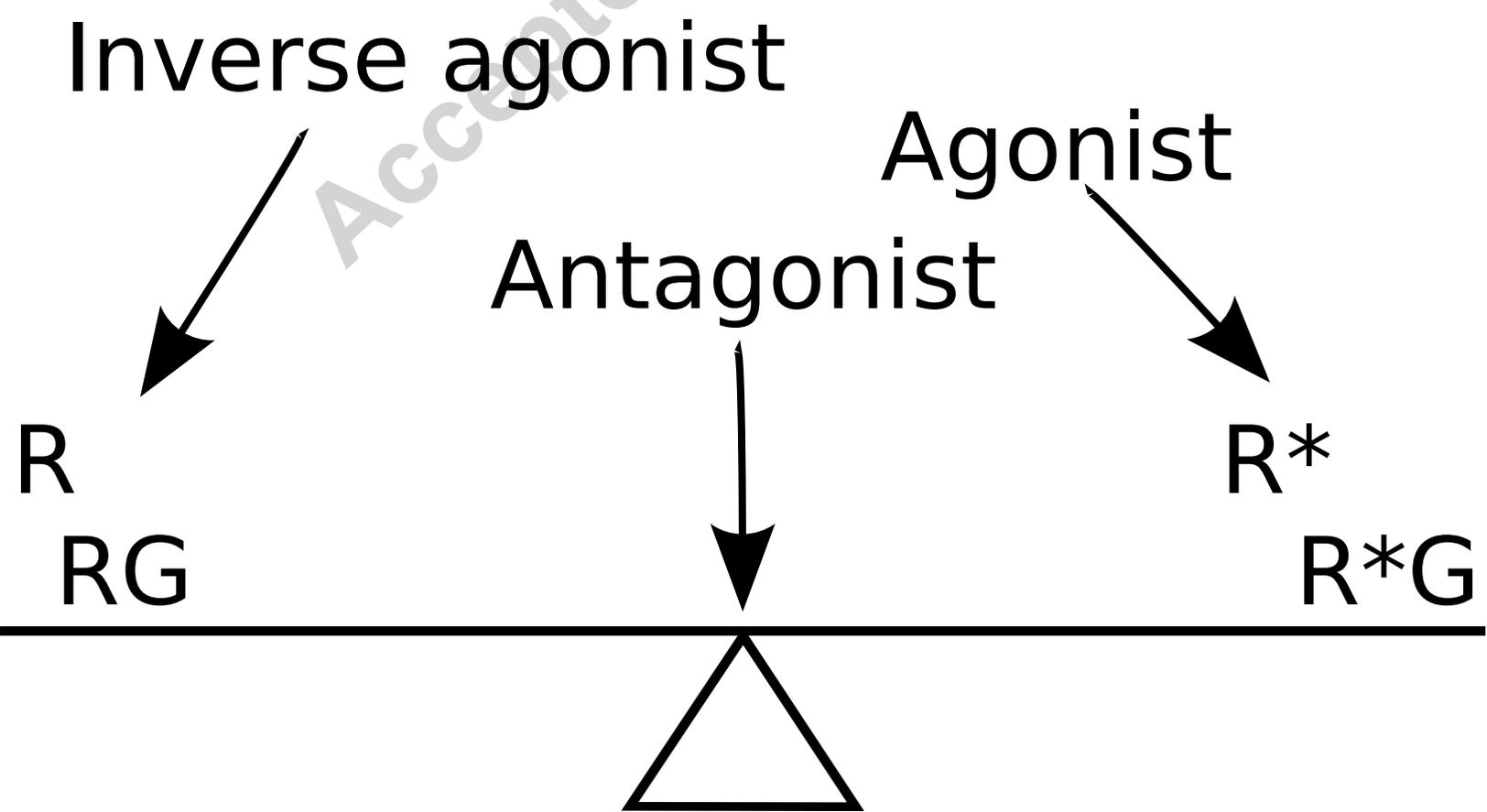
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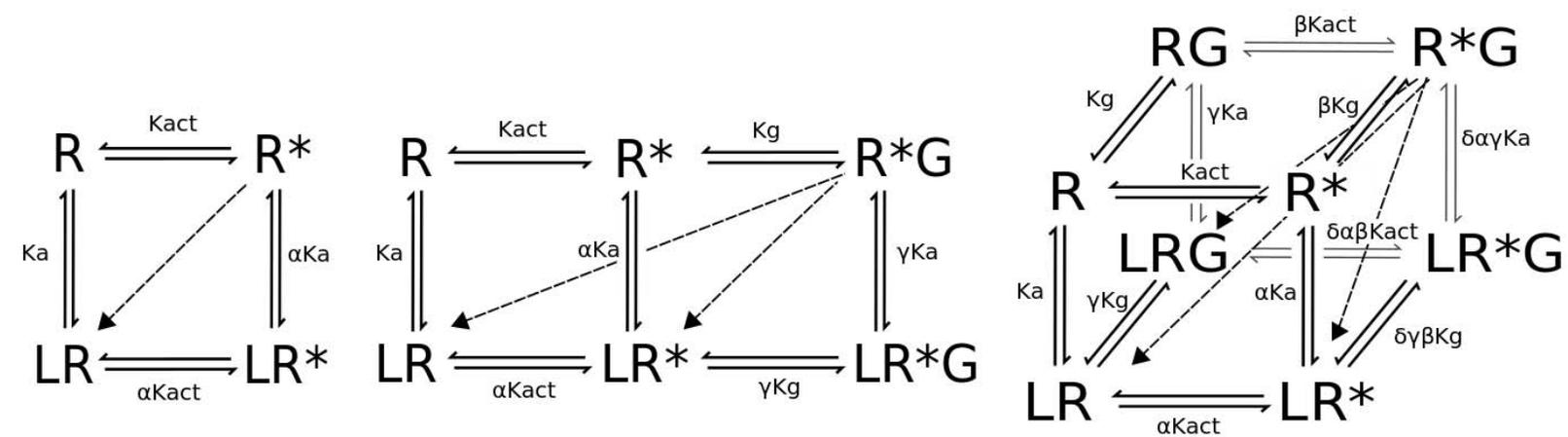
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Accepted manuscript



Inverse agonist

