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How inverse can a neutral antagonist be? Strategic questions after the rimonabant issue

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Rimonabant is an anti-obesity agent, at the therapeutic level, and a cannabinoid-1 receptor inverse agonist, at the molecular pharmacology level. The drug is currently off the market after psychiatric disorders were observed in some patients. If the adverse effects are attributed to its inverse agonist character, it makes sense to limit the drug discovery space to neutral antagonists. But do neutral antagonists exist? Here, the influence of the sensitivity of the signal transduction machinery on potential neutral antagonist misclassification is modelled. It is proposed that absolute neutral antagonists do not exist, and it is suggested that decisions about the continuity of the compounds in the drug development process be made in a quantitative inverse agonist scale rather than in a qualitative neutral antagonist and inverse agonist classification.

Rimonabant is an anti-obesity agent [1–4] whose sales were recommended to be suspended by the European Medicines Agency in October 2008 after some observed psychiatric disorders [5,6]. The clinical behaviour of a drug is the result of a sum of interrelated effects that derive, in the first instance, from the chemical structure of the compound and, in the second instance, from its pharmacological profile, both pharmacodynamic and pharmacokinetics. It is the pharmacodynamics and pharmacokinetics aspects related to the concept of antagonism that are the subject of this article.

From the molecular pharmacology side, rimonabant is a cannabinoid-1 receptor (CB1R) inverse agonist. There is no evidence that it is the inverse agonist feature of rimonabant that causes its side-effects; in fact, there are several (non-CB1R) G-protein-coupled receptor (GPCR) inverse agonists on the market wherein their inverse agonist behaviour does not lead to toxicity or side-effects. However, whole organism and molecular spaces are not completely independent scenarios and an association between undesirable side-effects (phenotype) and the inverse agonist label (genotype) seems plausible [7]. As a consequence, many investigations were reconducted towards receptor blockers lacking inverse agonism features; that is to say, towards neutral antagonists [8]. Yet the point is: do neutral antagonists actually exist?

By definition, a neutral antagonist is a ligand that binds all the existing conformations of a receptor with the same affinity and, because of that, does not change its basal response. This proposition (although useful as a theoretical construction to separate inverse agonists from agonists) seems unfeasible in practice because, thinking in molecular terms, two different ligand–receptor complexes – for instance, the inactive, *AR*, and the active, *AR** – are not likely to yield the same energy of formation. What would be expected is that the apparent neutral antagonist were, in fact, a partial agonist or a partial inverse agonist with such low efficacy that the generated signal would not be detectable by the technology used. Obviously, an increase in the sensitivity of the technique would reveal the true pharmacologic nature of the apparent neutral antagonist (see Ref. [9] for an adaptation to inverse agonists of classical methods [Schild and Cheng-Prusoff] for the estimation of antagonist affinity constants). As is shown below, the magnitude of the observed effect depends on both the signal transduction machinery of the cell and the capacity of the technique to change a signal input into a signal output. In the present

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study, two processes will be considered: the transformation of inactive receptors into active receptors and the functional translation of active receptor into observed effect. The magnitude of the latter step can depend on the functional assay performed.

It is worth noting that the assignment of the inverse agonist label to compounds previously considered neutral antagonists has already happened in the recent past when, after the functional determination of the first compound acting as an inverse agonist [10], many traditional neutral antagonists have been shown to be inverse agonists [11]; in particular, this is also true for most CB1R antagonists [12]. Caution is needed, however; although signal amplification can lead to distinguishing between inverse agonists of different efficacies, an excess of amplification might saturate the signal precluding the differentiation between ligands.

In this study, I do not discuss the following issues because they have been extensively addressed in recent reviews: (i) which should be the characteristics of suitable patients for a CB1R antagonist study [13,14]; (ii) the proposal of the peripheral versus the central site of action of CB1R antagonists and inverse agonists to avoid the undesirable effects resulting from the brain penetration of the compounds [15,16]; (iii) whether it is really important from the clinical point of view to assess whether and 'how much' rimonabant is an inverse agonist *in vivo* because there is now ever-increasing evidence that this compound acts against obesity by counteracting an increased tone of endocannabinoids both in brain and in periphery [17]; in the presence of increased levels of endogenous agonists, the distinction between inverse agonists and neutral agonists, if even they exist, would become difficult to assess *in vivo*; (iv) molecular aspects of the CB1R signal transduction [18]; (v) novel molecular approaches followed for the design of rimonabant analogues [19] and, a step forward, the synthesis of allosteric modulators of the CB1R [19,20]. Instead, I focus on a particular aspect of the functional response: the effects on the signal arising from changes in the system; for instance, the receptor density or the efficacy of the signal transduction process, and their consequences on the neutral antagonist and inverse agonist classification.

The observation depends on the observer's eyes

Inverse agonism at GPCRs was first proposed by Costa and Herz to designate the negative intrinsic activity of a compound binding to δ opioid receptors [10]. This property can be accounted by several pharmacological models of different complexity, which might include – among other components – the explicit presence of G proteins [11] and the arrangement of the receptor in dimeric states, either with equivalent protomers [21] or allowing for the possibility of asymmetry between the dimer subunits [22]. It is worth noting that GPCR functioning has become much more complex than initially thought. Indeed, even their name (G-protein-coupled receptors) is questioned, and a more general 'seven-transmembrane receptors' title has been proposed because they can signal through pathways involving not only G proteins but also other accessory proteins, such as β-arrestins, tyrosine kinases and PDZ-domain-containing proteins [23]. This versatility has led to the concept of functional selectivity, in which an inverse agonist would be any ligand that binds with higher affinity to conformations other than that responsible for the measured functional response [24]; these competitive conformations would include both inactive and active, the latter conformations involving pathways other than that selected for the study [25]. In the particular case of CB1Rs, it is well known that these receptors couple to adenylate cyclase (mainly via Gi/o but also via Gs), control ion channels and activate MAP kinases. It is also known that depending on the ligand, one transduction pathway can be favoured over the others. Thus, a compound could be a full inverse agonist at one transduction pathway and act as a barely detectable partial inverse agonist on another pathway [18]. This means that any discussion on drug efficacy should be made on a pathway-dependent basis [26]. Yet, for the purpose of the present study, it seems unnecessary to make use of a high degree of complexity to model the ligand–receptor interactions, and the two-state model of agonism seems to be sufficient.

The two-state model of agonism [27] considers two conformations, or states, for the free receptor: the inactive *R* and the active *R**. Ligands are classified as agonists, neutral antagonists or inverse agonists depending on their higher, equal or lower affinity for *R** relative to *R*, respectively (Box 1). Fig. 1 displays concentration–effect curves for two ligands, a pure neutral antagonist and an inverse agonist, in two conditions, (i) lower ($K_E = 10^{-7.5}$) and (ii) higher ($K_E = 10^{-9}$) system efficacy for the transduction of receptor activation into response. The neutral antagonist, as expected, does not change the basal response, which is higher for the more efficacious system, and a horizontal line for the functional response is obtained. The inverse agonist, by contrast, yields two very different results: in case (i), a curve practically indistinguishable from horizontal is obtained, giving the false impression that the compound is a neutral antagonist; in case (ii), increasing the sensitivity of the system allows the experiment to unmask the inverse agonist nature of the compound. Eq. (5) in Box 1 shows, through the operational efficacy τ parameter, that the same effect can be obtained by modifying either the signal transduction sensitivity of the system (K_E) or the level of receptor expression [R_T]. Decreasing K_E or increasing [R_T] increases τ and, as a consequence, amplifies the observed effect. The contrary happens by increasing K_E or decreasing [R_T]. It is worth noting that amplification of the signal through [R_T] or K_E is obviously relevant in *in vitro* systems. However, these effects can be neutralized in *in vivo* systems, in which both K_E and [R_T] will vary depending on the tissue and the physiopathological situation. This is an additional difficulty to take into account in translational studies, considering that the endocannabinoid system involves multiple functions in brain (striatum, hippocampus, cerebellum, cortex, hypothalamus and nucleus accumbens, among others regions, are involved) and in periphery (CB1Rs are expressed in adipose, skeletal muscle, liver, gastrointestinal tract, pancreas and other organs and tissues) [28].

Fig. 1 also shows the influence that ligand concentration can have on the conclusions for ligand classification. The separation between the curves for neutral antagonist and inverse agonist increases as the ligand concentration increases, this effect being greater as the system efficacy is higher. It can be said that no neutral antagonist remains 'neutral' at high doses, compatible with its solubility.

Functional and therapeutic issues are connected

The drug discovery and development process is a complex task in which an enormous investment is needed for a not guaranteed successful result [29]. Because of this, a reported negative outcome in a particular laboratory has immediate consequences on all the pharmacology community working on the same or a related project. Coming back to the rimonabant issue, a question arises: should a research project on CB1Rs be cancelled because a compound initially considered to be a neutral antagonist shows, at some point during its development, an inverse agonist behaviour? Had the inverse agonist behaviour of rimonabant been responsible for its collateral psychiatric disorders, the answer would be yes; but it could be also true that it is its negative efficacy that makes rimo-

BOX 1

The two-state model of agonism

The receptor disposes of two states or conformations, an inactive (*R*) and an active (*R**), the relative populations of which in the absence of ligands are governed by the equilibrium constant *X* (Figure I). The preferable binding of a ligand to *R** or *R* makes the ligand an agonist or an inverse agonist, respectively. In the case of a ligand with equal affinities for both states, the ligand is called a neutral antagonist. If the biological response is attributed to *R**, agonists and inverse agonists increase and decrease the basal response, respectively, whereas neutral antagonists have no effect on it (see Ref. [27] for review and Ref. [30] for notation).

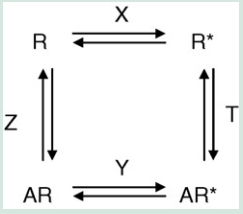

FIGURE I

The two-state model of receptor agonism.

The equilibrium constants are defined as:

$$X = \frac{[R*]}{[R]};\ Y = \frac{[AR*]}{[AR]};\ Z = \frac{[A][R]}{[AR]};\ T = \frac{[A][R*]}{[AR*]} \quad (1)$$

and the total receptor concentration is:

$$[R_T] = [R] + [R*] + [AR] + [AR*] \quad (2)$$

In terms of the equilibrium constants, $Y > X$ and $T < Z$, for agonists; $Y < X$ and $T > Z$, for inverse agonists; and $Y = X$ and $T = Z$, for neutral antagonists. The $Y{:}X = T{:}Z$ ratio defines the intrinsic efficacy of the ligand, that is, the capacity of the ligand for the transduction of receptor occupation into receptor activation. The fraction of active receptors is:

$$f_{R*} = \frac{[R*] + [AR*]}{[R_T]} = \frac{T + [A]}{Ta + b[A]} \quad (3)$$

where $a = 1 + 1/X$ and $b = 1 + T/(XZ)$. Parameters a and b have mechanistic interpretation; $1/a$ is equal to f_{R*} in the absence of ligand (related with constitutive receptor activity) and $1/b$ is equal to f_{R*} for large ligand concentrations (related with intrinsic ligand efficacy). Parameters a and b both range between 1 and $+\infty$. The value of one yields the maximum value for f_{R*} (i.e. one), whereas large values for these parameters produce low values for f_{R*} (close to zero).

Signal transduction is made of a succession of processes in which the output of the former constitutes the input of the latter. If we consider that receptor activation is the input for a global process leading to the observed effect (E), we can write the following expression, which makes use of the operational model of agonism formalism [31]:

$$E = \frac{E_m([R*] + [AR*])}{([R*] + [AR*]) + K_E} \quad (4)$$

where E_m is the maximum possible effect and K_E is the intrinsic efficacy of the system for the transduction of receptor activation into response. Note that, following a parsimony criterion, it is assumed in Eq. (4) that the free (*R**) and the ligand-bound (*AR**) active receptor species have the same capacity for signal transmission.

If we divide the numerator and denominator of Eq. (4) by $[R_T]$, we obtain

$$E = \frac{E_m f_{R*}}{f_{R*} + 1/\tau} = \frac{E_m(T + [A])/(Ta + b[A])}{(T + [A])/(Ta + b[A]) + 1/\tau} \quad (5)$$

where $\tau = ([R_T]/K_E)$ is the operational efficacy, that is, a system parameter for the transduction of receptor activation into physiological effect. We see that τ is the combination of two parameters: one, $[R_T]$, resulting from the level of receptor expression and the other, K_E, from the signal transduction capacity of the system.

Eq. (5) shows the dependence of the observed effect with ligand concentration. For large ligand concentrations, Eq. (6) is obtained.

$$\lim_{[A]\to\infty} E = \frac{E_m}{1 + b/\tau} \quad (6)$$

We see that the asymptotic value observed for E, which will determine the classification of the ligand as agonist, neutral antagonist and inverse agonist, depends on the ligand intrinsic activity for translating ligand-bound receptors into activated receptors (through parameter b) and on the operational efficacy for translating active receptors into pharmacological response (through the parameter τ).

nabant useful as an anti-obesity agent [28]. A solution to this dilemma, at least in part, might be to focus attention on the quantitative, rather than the qualitative, profile of the compounds. That is to say, if a previously found neutral antagonist acts as an inverse agonist in a higher sensitive assay, it will be how large the difference in efficacy is relative to rimonabant rather than the qualitative character of this property that can give us the right arguments for the evaluation of the risks associated with the continuity of the project. As mentioned above, CB1Rs are coupled to more than one signalling pathway. Thus, any assay designed as a stop-or-go decision for the development of a CB1 drug should be proposed

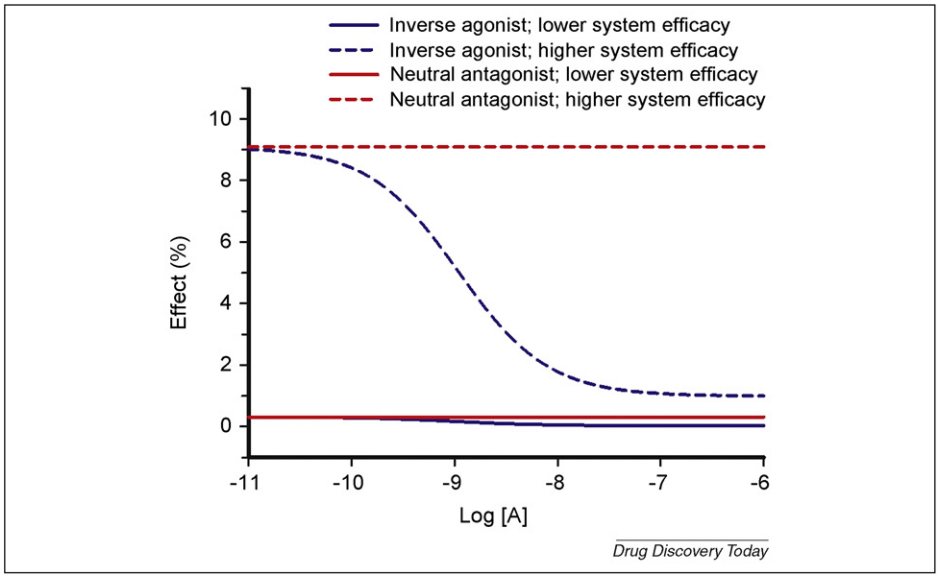

FIGURE 1

Simulation of the physiological effect exerted by a neutral antagonist (red line) and an inverse agonist (blue line) in lower ($K_E = 10^{-7.5}$) and higher ($K_E = 10^{-9}$) efficacy systems (solid and dashed lines, respectively). Fixed parameter values: $E_m = 100$; $[R_T] = 10^{-4}$; $X = 10^{-6}$; neutral antagonist: $T = 10^{-9}$, $Z = 10^{-9}$; inverse agonist: $T = 10^{-8}$, $Z = 10^{-9}$.

for a specific pathway. In this regard, a pathway-specific standardized scale of efficacies would be helpful. To properly compare between laboratories, an agreement on the compound acting as an internal reference would be needed.

Concluding remarks

In this article, the clinical failure of the CB1R inverse agonist rimonabant is used to discuss some general issues regarding inverse agonism versus neutral antagonism. It is worth noting that the reason for cancelling the clinical use of rimonabant was its psychiatric side-effects, and this decision would be the same notwithstanding the pharmacology profile (neutral antagonism or inverse agonism) of the compound. Nevertheless, some connections can be expected between molecular and therapeutic levels, which might putatively suggest that inverse agonism can be an issue for CB1R anti-obesity research. There can be many reasons to stop a drug candidate (toxicity, poor pharmacokinetics, lack of clinical efficacy, and so on) but, in addition, the molecular aspect of inverse agonism is probably a particular concern in the mind of many investigators working in the CB1R field.

To consider neutral antagonists as those drugs to address, CB1R pharmacological research needs first to establish whether these molecules do occur. Accepting that ligands are unlikely to display the same affinity for different receptor states leads to the conclusion that neutral antagonism is a concept that probably does not exist in real world; therefore, apparent neutral antagonists are probably inverse agonists that have not yet been unmasked. Amplification of the signal by increasing the operational efficacy of the system (decreasing K_E or increasing $[R_T]$) means that an apparent neutral antagonist behaves as an inverse agonist. In this line of thought, it is important to remark that because the pharmacological profile of a ligand depends on system conditions, comparisons between ligands should be done under the same system parameters and consistency among series of experiments would be required.

Finally, it is suggested that rather than a qualitative distinction between neutral antagonists and inverse agonists, a quantitative analysis of the magnitudes of inverse agonist efficacies should be done before any decision concerning the continuity of a project after a neutral antagonist and inverse agonist change in the pharmacologic classification of a drug.

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