CNS Spectrums (2019), **24**, 349–351.
doi:10.1017/S1092852918001761

EDITORIAL

Should inverse agonists be defined by pharmacological mechanism or clinical effect?

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Received 19 November 2018; Accepted 18 December 2018; First published online 20 March 2019

Pimavanserin was recently approved for treating hallucinations and delusions associated with Parkinson's disease.[1] It is the first approved drug that selectively targets the 5-HT_{2A} receptor and is termed a selective serotonin inverse agonist. This has motivated a debate about which drugs should be classified as inverse agonists – those that display clear evidence of inverse agonist activity *in vitro*, or only those that have demonstrated clinical evidence of inverse agonism.[2] This is a valid question. Here I advance the view that drugs are defined by their *in vitro* pharmacological profiles and mechanisms of action to the extent that these mechanisms are understood. Constitutive receptor activity and inverse agonism are now well-defined pharmacological properties, documented at the structural level. Therefore, calling pimavanserin an inverse agonist based on its molecular pharmacology properties is appropriate.

Definitions

Constitutive activity or basal activity is defined as receptor-mediated activity in the absence of agonists. Receptors are flexible proteins and undergo spontaneous conformational changes between inactive conformations and active conformations capable of signaling. This model is formally identical to the allosteric model of enzyme activation.[3] In this context, agonists increase receptor activity by stabilizing active conformations, and inverse agonists suppress receptor activity by stabilizing inactive conformations.[4]

Antagonists are defined as compounds that reduce the receptor-mediated responses to other compounds. Commonly this means blocking the actions of an agonist by competing for the same binding site (competitive antagonism), although other forms of antagonism also exist (e.g., allosteric antagonism). In practice, many antagonists are also inverse agonists because they both suppress spontaneous or constitutive receptor activity and block actions of agonists. This is the case for pimavanserin.

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Structural Basis

The theoretical model for receptor constitutive activity and the actions of agonists and inverse agonists have all been captured in fine structural detail. Excellent examples can be found in a recent paper from Manglik *et al.*[5] These studies employed ^{19}F-fluorine NMR and double electron-electron resonance spectroscopy to visualize the structural dynamics of spontaneous conformational changes of the β2 adrenergic receptor (β2AR) and interactions of β2AR receptors with agonists, inverse agonists, and G-proteins.

Manglik *et al.* showed that there are at least two forms, each of active and inactive conformations (see Figure 6A in ref. [5]). Furthermore, they observed that an inverse agonist destabilized the active conformations and stabilized two rapidly interconverting inactive conformations. They further described two active conformations, a partially active conformation that was promoted by agonist binding and a fully activated conformation that was stabilized by both an agonist and the G-protein. These results are consistent with the concept of spontaneous transitions occurring between inactive and active conformations, with inverse agonists favoring inactive conformations, and agonists and G-proteins promoting active conformations.

Pharmacological Consequences

A variety of pharmacological data are explained by these structural observations. It is known that mutations often constitutively activate G-protein-coupled receptors (GPCRs). These observations validate the concept that constitutive activity is an intrinsic property of GPCRs, and that many GPCR ligands are inverse agonists. In a

classic study, all 19 possible amino acid substitutions for the wild-type alanine at position 293 constitutively activated the α1b adrenergic receptor.[6] Since such divergent amino acid substitutions all raised constitutive activity, it was inferred that the mutations disrupted a "constraining function" of that region of the receptor, releasing spontaneous receptor activity. This would only happen if there was a natural tendency for the receptor to adopt active conformations.

We reached a similar conclusion from a series of studies employing large-scale random mutagenesis that showed that constitutively activating mutations (CAMs) are common, are clustered in key conformational "switch" regions, and divergent classes of amino acid substitutions are capable of constitutively activating the M5 receptor, similar to what was observed with the α1b adrenergic receptor.[7] Together, these studies support the concept of receptors as flexible proteins that interconvert between inactive and active states, which in turn can be promoted by inverse agonists and agonists, respectively.

Another important implication from the structural studies described above is that G-proteins would be expected to stabilize active conformations of GPCRs. This predicts that GPCRs coupled to G-proteins would have higher affinity for agonists than uncoupled GPCRs; that overexpressing G-proteins would promote the constitutive activity of GPCRs; and that inverse agonists would suppress G-protein-induced constitutive activity – all of which have been demonstrated experimentally.[8]

Constitutive Activity of GPCRs, 5-HT_{2A} Receptors, and Inverse Agonist Activity of Pimavanserin

Using a proprietary high-throughput functional assay called Receptor Selection and Amplification Technology (RSAT™) that is very sensitive to differences in the constitutive activity of GPCRs, we evaluated well over 100 GPCR targets during the course of our drug discovery efforts.[9] These assessments were carried out with wild-type, unmutated receptors. We observed a tremendous variability in the degree of constitutive activity for each receptor, ranging from very low (H1 histamine, 5-HT_{2B}) to very high (5-HT_{2C}).[10] Where comparisons are possible, our findings correlated with observations of others. Receptors with high constitutive activity in RSAT™ were receptors for which others observed constitutive activity in native systems or *in vivo*, such as the M2 muscarinic receptor,[7] the ghrelin receptor,[8,11] and the 5-HT_{2C} receptor.[10,12] Compared to all of the receptors we tested, the 5-HT_{2A} receptor has constitutive activity levels ranking approximately in the top third, below the levels we saw for 5-HT_{2C} and ghrelin, but well above many other receptors such as the 5-HT_{2b} serotonin receptor (Burstein, unpublished observations).

Several reports describe the constitutive activity of 5-HT_{2A} receptors *in vivo* in regulating the responses to an associative learning test – the acquisition of the rabbit's learned eyeblink response (extension of the nictitating membrane) to a conditioned stimulus.[12] The reflexive or motor response to an unconditioned stimulus was also recorded. The authors described three classes of 5-HT_{2A} drugs: agonists that enhanced learning, antagonists that had no effect on learning, and inverse agonists that impeded learning. Moreover, they demonstrated that the antagonists could block the effects of both agonists and inverse agonists in this paradigm. To confirm the genuine constitutive activity of 5-HT_{2A} receptors, these experiments were successfully repeated with depletion of endogenous 5-HT by the neurotoxin 5,7-dihydroxytryptamine.[13]

We profiled many of the compounds described in ref. [12] in RSAT™. All of the compounds we identified as inverse agonists in RSAT™ (M100907, Ritanserin, Mianserin, SR46349N, Ketanserin) were inverse agonists in the learned eyeblink test, and all but Ketanserin were inverse agonists in the motor response. Two compounds, d-Bromolysergic acid diethylamide (BOL) and LY-53,857, were identified as pure antagonists in both the learned and motor eyeblink response. We did not profile BOL but found that LY-53,857 behaves like a pure 5-HT_{2A} antagonist in RSAT™ in that it blocks the effects of both agonists, such as 5-carboxytryptamine, and inverse agonists, such as ritanserin and pimavanserin, but has little intrinsic activity of its own (Burstein, unpublished observations).

Recently a number of valid issues have been raised regarding extrapolating the clinical relevance of findings from *in vitro* pharmacology experiments. Using slow wave sleep (SWS) as an example, the difficulties of distinguishing the *in vivo* actions of 5-HT_{2A} inverse agonists from 5-HT_{2A} antagonists have been noted.[2] The attempt to correlate SWS with inverse agonism is confounded by the fact that many of the compounds listed in Table 1 of Nutt *et al.* are not selective, and interact with other receptors that may affect SWS. There are published reports distinguishing 5-HT_{2A} inverse agonism from antagonism *in vivo*, suggesting that these different *in vitro* profiles may have meaningful consequences *in vivo*.[12] Despite this report, the physiological impact of the constitutive activity of 5-HT_{2A} receptors *in vivo* is still not well understood, and thus it is difficult to say what clinical differences may exist between 5-HT_{2A} inverse agonists and pure antagonists. Further work is warranted to understand the biological significance of inverse agonism at 5-HT_{2A} receptors. Nevertheless, our position is that pimavanserin is appropriately defined by its pharmacological properties, which are consistent with the most current understanding of GPCR pharmacology. The term "inverse agonist" is a pharmacological term that

https://doi.org/10.1017/S1092852918001761 Published online by Cambridge University Press

accurately describes the pharmacological properties of pimavanserin, and that is our basis for designating pimavanserin as a selective serotonin inverse agonist.

Disclosures

Dr. Burstein is an employee of ACADIA Pharmaceuticals, Inc.

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https://doi.org/10.1017/S1092852918001761 Published online by Cambridge University Press