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COMMENTARY

What systems can and can't do

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This commentary discusses a paper in this issue by Dr Jillian Baker on the antagonism of histamine H_2 receptors. It is an excellent example of the use of pharmacological principles to determine what systems can and can't do from the point of view of agonist-dependent antagonism. The most common model of antagonism, namely orthosteric, cannot discern agonist type; i.e. all agonists are blocked equally by a given orthosteric antagonist. Therefore, if quantitative assessment of antagonism unveils agonist dependence, then this is something an orthosteric mechanism cannot do and another mechanism must be considered. A simple alternative is a permissive allosteric model whereby the agonist and antagonist interact through conformational changes in the receptor protein. Under these circumstances, an agonist–antagonist dialogue can ensue whereby the nature of the agonist determines the magnitude of antagonist effect. Jillian Baker contrasts antagonist systems with historical data obtained for β-adrenoceptors and the present data for histamine H_2 receptors where the simpler model of orthosteric antagonism suffices and thus shows how quantitative receptor pharmacology can be used to determine the molecular mechanism of antagonism.
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A focus of what has been termed 'receptor pharmacology' is the application of models to drug behaviour. Although not always formally acknowledged, every time experimenters picture in their mind's eye what happened in their experiment, consciously or unconsciously they have formulated a model. However, the best models are those with defined rules and these usually involve simple mathematics that allow quantitative predictions to be made. The importance of such rules is that they define what a given system can and cannot do, and when the latter condition is encountered, the model can be modified and a presumably more accurate picture of drug mechanism emerges. From the pioneering work of pharmacologists such as Gaddum, Ariens, Stephenson, Schild, Van Rossum, Furchgott, Paton, Waud, Rang and MacKay (Rang, 2006), a theoretical framework for drug behaviour in cellular systems that has formed the basis for pharmacological taxonomy of receptors and drugs for the past 60 years has come. A fundamental building block of these systems is the orthosteric interaction of agonists and antagonists with receptors. The key feature emerging from the models describing antagonism is that the systems are pre-emptive, that is, an antagonist pre-empts agonist action with no regard for the identity of that agonist. This can be observed from an examination of the equations describing antagonism with these models that have no cross terms involving agonist and antagonist, only separate ones, thereby precluding modification of antagonist effect with different agonists. Thus, a given antagonist will be absolutely equipotent for all agonists if equilibrium conditions are attained in the system.

These orthosteric models formed a robust framework for drug classification in spite of the fact that seven transmembrane receptors are Nature's prototype allosteric protein, binding ligands in one region to change their shape and affect protein–protein interactions in other regions. The allosteric nature of receptors has not been overlooked in formulations of receptor theory as shown by pioneering papers by Karlin (1967), Colquhoun (1973), Thron (1973), Ehlert (1988, 2005) and Leff *et al*. (1997). However, with the exception of muscarinic receptors, early examples of allosteric antagonism in receptors were sparse (perhaps a function of the orthosteric bias introduced by radioligand screening at the time; Rees *et al*., 2002). Thus, by far, the bulk of evidence has suggested that antagonists functioned as off switches for receptors with little texture between them other than pharmacokinetics, temporal offset kinetics and potency.

Technological revolutions in pharmacology have changed all that and, presently, the increasing ability to screen for new ligands in functional modes and new eyes to see the signalling effects of agonists and antagonists have revealed ever-increasing ligand-based allosterism. First noted for agonists (alternatively referred to as 'stimulus trafficking', 'stimulus-bias', 'ligand-based functional selectivity'—for review, see special issue *Trends Pharmacol Sci*, August 2007),

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presently there is less prevalent texture in antagonism reported. However, examples are increasingly being noted (Baker and Hill, 2007; Bosier and Hermans, 2007; Leach *et al*., 2007) of antagonists that 'edit' the signals produced by endogenous agonists. For example, the natural agonist neurokinin A activates G_s and G_q through NK_2 receptors, whereas the allosteric modulator LP1805 changes this pattern to one of the enhanced G_q and antagonism of G_s activation (Maillet *et al*., 2007). Similarly, the allosteric modulator AMD3100 blocks CXCR4-mediated chemotaxis by the natural agonist SDF-1α but not the effects of peptide fragments RSVM and ASLW (Sachpatzidis *et al*., 2003). CRTH2 receptors interact with G_i and β-arrestin on activation by the natural agonist prostaglandin D_2; on binding of the modulator Na-Tosyltryptophan, prostaglandin D_2 loses its ability to cause β-arrestin interaction but still causes activation of G_i (Mathiesen *et al*., 2005). Such pharmacological annotation is beyond the capability of an orthosteric pre-emptive antagonist mechanism (Arunlakshana and Schild, 1959) and thus, when such behaviour is observed, another mechanism must be auditioned for the part. One such mechanism is permissive allosteric antagonism whereby the antagonist binds to a site different from the agonist and modifies the actions of that agonist through conformational changes in the receptor protein. Within this frame of reference, allosteric antagonists form permissive systems whereby the identity of the agonist matters to the antagonist in accordance with the known probe dependence of allosteric systems (Kenakin, 2005). This concept is evident in a number of allosteric models whereby cross terms exist for both agonist and antagonist with terms quantifying their relationship (Hall, 2000; Christopolous and Kenakin, 2002; Ehlert, 2005; Kenakin *et al*., 2006; May *et al*., 2007).

In this issue, Baker (2008) gives an excellent overview and history of this phenomenon through published data on β-adrenoceptors (Granneman, 2001; Baker *et al*., 2003; Baker, 2005a, b). If, for the sake of argument, a conventional orthosteric mechanism can be considered more simple than an allosteric two-site system, then standard hypothesis testing can be used to discern mechanisms of antagonism, that is, if observations are made that are incompatible with the most simple scheme, then it is rejected as a possibility and another possibility is sought. Dr Baker shows a way forward for the taxonomy of antagonism for receptor mechanisms in general through the use of careful receptor-based null pharmacology to test consistency with standard orthosteric antagonism. On the basis of the notions of what systems can and cannot do, the default is to assume the simplest (pre-emptive) model until heterogeneity in antagonism is observed. A simple competitive mechanism cannot accommodate heterogeneity in antagonism, such as that described by Baker for β-adrenoceptors. This reasoning is applied in this present paper to histamine H_2 receptors where the data indicate that the simple models suffice (Baker, 2008). A valuable message in this paper is the power of null functional methods in quantitative pharmacology. An intriguing prospect within this analysis is the notion that a receptor system may be prone towards pre-emptive vs permissive kinetics. Although it may be too early to draw this conclusion, the existing evidence supports this some receptors appear to be allosterically inclined with respect to antagonism (that is, muscarinic), whereas others (in this case, histamine) may not. However, it may also be difficult to make these classifications as a rule is a rule until it is broken, that is, in this case, until an allosteric H_2 receptor antagonist that shows the type of heterogeneity seen for β-adrenoceptors, is found. With the increasing opportunity to screen allosteric modulators, it will be interesting to see if receptor types emerge. In general, these data show the power of functional receptor pharmacological methods in understanding complex ligand interaction at receptors. This process is very important as it can classify antagonists as orthosteric or allosteric, labels that carry with them different behaviours and capabilities in therapeutic systems.

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