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COMMENTARY

Supra-physiological efficacy at GPCRs: superstition or super agonists?

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The concept of 'super agonism' has been described since the discovery of peptide hormone analogues that yielded greater functional responses than the endogenous agonists, in the early 1980s. It has remained an area of debate as to whether such compounds can really display greater efficacy than an endogenous agonist. However, recent pharmacological data, combined with crystal structures of different GPCR conformations and improved analytical methods for quantifying drug action, are starting to shed light on this phenomenon and indicate that super agonists may be more than superstition.

LINKED ARTICLE
This article is a commentary on Schrage *et al.*, pp. 357–370 of this issue. To view this paper visit http://dx.doi.org/10.1111/bph.12003

In the accompanying paper, Mohr and colleagues have identified iperoxo and closely related analogues as agonists with higher efficacy at the muscarinic ACh M_2 receptor than the endogenous agonist, ACh (Schrage *et al.*, 2013). Using label-free dynamic mass redistribution assays in CHO cells stably expressing the muscarinic M_2 receptor, supplemented with [^{35}S]GTPγS assays of activated G proteins, iperoxo was validated as a highly potent agonist, with a similar maximal effect to that of ACh. However, analysis according to the operational model of agonism (Black and Leff, 1983) revealed that iperoxo has a higher estimated operational efficacy (τ) value than the cognate agonist. Furthermore, inactivation of the muscarinic M_2 receptor population using the irreversible antagonist phenoxybenzamine, followed by assessment of receptor function, directly indicated that the maximal response to ACh was more susceptible to receptor alkylation than those observed for iperoxo, again indicating that the latter possesses higher intrinsic efficacy than the former. Key conclusions from this study are the suggestion that GPCRs have not evolved to ensure maximal activation at the molecular level by their cognate agonist, and a proposal to more frequently incorporate analytically derived parameters, specifically operational measures of agonist efficacy, routinely into drug discovery programmes.

Although not common for GPCRs, previous reports of super agonism have been described in the literature for peptide hormone GPCRs (Loumaye *et al.*, 1982) and other receptor families – notably for the CD28 receptor (Tacke *et al.*, 1997; Farzaneh *et al.*, 2007). The term 'super agonist', although not formally recognized by the Nomenclature Committee of the International Union of Basic and Clinical Pharmacology (Neubig *et al.*, 2003), has historically been used to describe the actions of compounds that elicit a maximal effect greater than that of the endogenous agonist(s) of a receptor. Such terminology has been met with mixed views in the pharmacology community, presumably because of the assumption by many that the interaction between an endogenous ligand and its receptor, as a result of strong evolutionary pressure, is likely to be as efficient as it can possibly be. However, at least with respect to GPCRs, there is no *a priori* reason why this should actually be the case. As shown in recent crystallographic studies of GPCRs (Audet and Bouvier, 2012; Katrich *et al.*, 2013), the presence of a G-protein or suitable mimic is required to stabilize the receptor in an active conformation; the presence of a highly efficacious agonist alone is insufficient to do so (Rasmussen *et al.*, 2011). Recent agonist-receptor co-structures do reveal some differences from their respective antagonist/inverse agonist-bound

states (as shown for both the adenosine A_{2A} receptor and β_1-adrenoceptor; Warne *et al.*, 2008; Doré *et al.*, 2011; Lebon *et al.*, 2011; Warne *et al.*, 2011). However, the conformational differences between the binary (AR) and ternary (ARG) complex structures of the β_1-adrenoceptor and opsin, particularly with respect to the outwards movements of transmembrane domains 5 and 6, are much larger still (Audet and Bouvier, 2012; Katrich *et al.*, 2013). Therefore, while the role of the agonist alone in defining the active state of the receptor is vital, it is certainly no more important than that of the G-protein. Moreover, theoretical considerations highlight that maximal degrees of GPCR activation are unlikely to be achieved at the level of the ligand–receptor complex (even by compounds classed as highly efficacious agonists in cellular or tissue systems) because of the high concentrations of guanine nucleotides in the cytosol that ensure the receptor is regularly driven away from the active state (see Ehlert, 2008); this is the 'natural' state to which both the receptor and its endogenous agonist(s) have adapted. Collectively, these observations indicate that GPCRs have substantial conformational freedom to achieve higher levels of activation, and thus synthetic 'super agonists' are likely to be more feasible than perhaps first thought.

A second important consideration that arises from these observations is the need to incorporate the analytical determination of agonist signalling efficacy into the drug discovery process in a manner that can assist structure-activity studies. Of course, this is not a new concept, but as alluded to by the authors of the accompanying paper, is certainly underutilized compared with the far more routine approach of relying on empirical potency or E_{max} parameters, which are capriciously system- and assay-dependent (Figure 1). This has taken on an extra degree of urgency in recent years because of the increased identification of signal pathway-biased agonists (*vide infra*). In the study presented by Schrage *et al.*, the authors chose the operational model of agonism (Black and Leff, 1983) as the analytical tool of choice to derive relative efficacy values (τ) for statistical comparisons, which led to the conclusion that iperoxo had higher efficacy than the cognate agonist, ACh. The advantage of the operational model is that it can be routinely applied to experimentally determined data under most assay conditions, provided complete concentration–response relationships are established for test agonists. A disadvantage, as is the case with any analytical method, is the robustness with which the model can be fitted to the data and the potential for researchers to over-interpret the meaning or reliability of the fitted parameters in the absence of additional 'checks'. As outlined in the accompanying paper, the authors used the equilibrium constants derived from radioligand binding studies as the K_A parameter in the operational model fits to the functional data. This method assumes that the affinity determined from a binding assay reflects the functional affinity operative at the level of the whole cell, which is often found not to be the case. Indeed, it is noteworthy the authors of the accompanying study themselves found a discrepancy between the

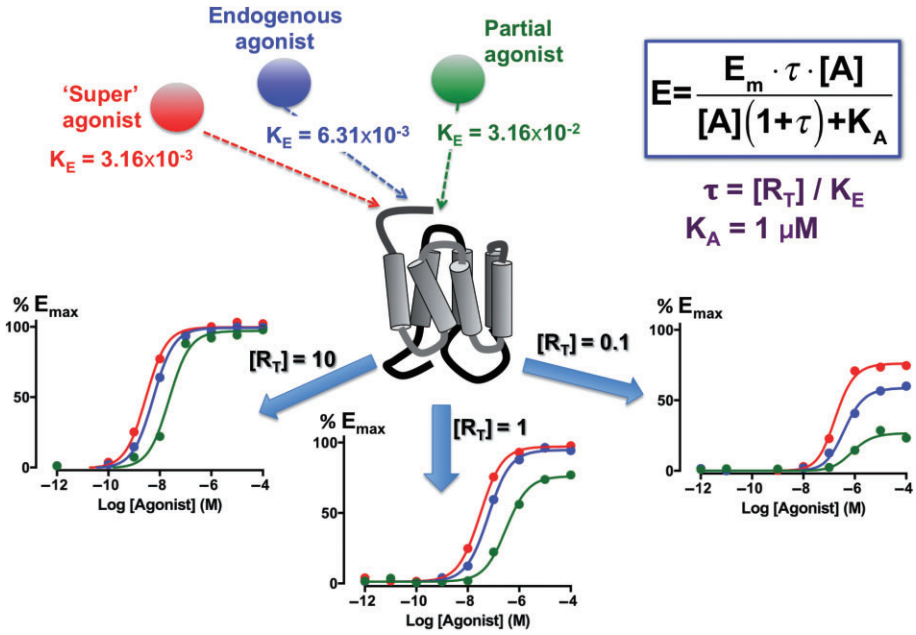

Figure 1

System dependence of observed agonism. Simulations using the operational model of agonism (box) of three agonists with varying efficacies, as indicated by the signalling efficacy parameter, K_E (arbitrary units). A 'super' agonist is defined as having a higher intrinsic efficacy (modelled with a smaller K_E value) than the cognate 'endogenous' agonist. Note that variations in receptor density (modelled as normalized [R_T] values) can have a profound effect on whether differences in maximal agonist responsiveness and, hence, direct demonstration of differences in agonist efficacies, will be observed; similar effects will be seen with variations in stimulus–response coupling efficiency. Appropriate analytical approaches, such as the operational model, can be used to provide insight into such differences via the determination of K_A and τ values. For the simulations, the value of E_m was 100. All other parameter values are as indicated in the Figure.

estimated τ values for G_s activation by ACh and the actual experimentally observed maximum agonist efficacy – likely because of the use of an inappropriate value for the operational K_A parameter. An alternative approach that the authors used to negate the need for potentially confounding effects of independently determining affinity values was irreversible alkylation of the receptors via the method of Furchgott (1966), thus reducing the receptor reserve and allowing differences in agonist signalling efficacy to be directly manifested as changes in the maximal agonist response. However, this approach is laborious and depends on the availability of a suitable alkylating agent, which may not be available for other GPCRs. A second potential method is to use the operational model to directly determine *all* the relevant parameters from the functional data at hand. This can certainly be done if agonist 'power' is determined as the *ratio* of τ/K_A, which has recently been shown to be a very useful parameter for quantifying biased agonism (Kenakin *et al.*, 2012), but not always possible if the goal is to separate τ from K_A for full agonists (it is relatively trivial to get both parameters separately for partial agonists). Irrespective, the simulations in Figure 1 illustrate how it can be difficult to differentiate high- from low-efficacy agonists (or 'super' from 'not-so-super' agonists) in systems possessing high levels of receptor expression or stimulus–response coupling; additional analytical and experimental manipulations must be utilized to make such distinctions in these instances.

If one accepts the notion that super agonism is feasible, it leads to important questions regarding the role of such molecules within GPCR pharmacology. Therapeutically, are there indications where a super agonist might be the desired product? Where the function of the receptor in question is 'excitatory' in nature, then such a molecule may *not* be desirable viz. the 'cytokine storm' initiated in humans by the CD28 super agonist, TN1412 (Farzaneh *et al.*, 2007). It is also easy to envisage how super agonists of post-synaptic, excitatory GPCRs in the CNS may lead to profound neurotoxic side effects. However, there are potentially GPCRs for which a super agonist may be desirable, especially where the receptor expression or function is impaired in a disease state or where the net function of an activated receptor is primarily inhibitory (e.g. pre-synaptic autoreceptors). Furthermore, as indicated by Mohr and colleagues, super agonists might prove very useful tools in facilitating structural and mechanistic studies of GPCRs.

Finally, one of the most interesting areas of current GPCR biology is the recognition that different agonists for the same receptor can direct signalling biased to particular pathway(s). The phenomenon is referred to as 'biased agonism', 'ligand-directed signalling' or 'functional selectivity' and is thought to arise as agonists engender multiple active state conformations that exist between the ground state of the receptor and the conformation defined by the endogenous agonist and effector proteins. If one assumes that biased agonists are able to promote a greater spectrum of receptor conformational states than 'non-biased' agonists, then one might expect such ligands to more readily manifest as partial agonists or antagonists/inverse agonists depending on the pathway investigated (because of their distribution across many receptor states). In contrast, compounds that enrich fewer receptor states may manifest as higher efficacy agonists (or inverse agonists) for those states. Therefore, it may be that super agonists, by virtue of their very high efficacy, may be less capable of sampling multiple active states and hence would be less biased. Although speculative, this is a notion that can be explored. Given the expanding field of chemical and structural biology, coupled with the analytical tools with which to dissect *in vitro* pharmacology, the identification of super agonists of GPCRs may become more prevalent in the future and open new pathways for understanding GPCR functionality.

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