BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01793.x
www.brjpharmacol.org

COMMENTARY

Allosteric modulators can restore function in an amino acid neurotransmitter receptor by *slightly* altering intra-molecular communication pathways

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Keywords
allostery; conformational selection; allosteric drug design; drug development; membrane receptors; disease-related mutations; induced fit; channel gates; protein-protein interaction networks; protein structure networks; signalling

Received
4 October 2011
Revised
8 November 2011
Accepted
16 November 2011

Mutations, even if not directly in the ligand binding sites of proteins, can lead to disease. In cell surface receptors, this can happen if they uncouple conformational changes that take place upon agonist (or antagonist) binding to the extracellular domain and the intracellular response. Uncoupling can take place by disrupting a major allosteric propagation pathway between the extra- and intracellular domains. Here I provide a mechanistic explanation: I first describe how propagation takes place; second, what can happen in the presence of a disease-related mutation which is distant from the binding site; and finally, how drugs may overcome this disruption and rescue function. The mutations in the glycine receptor α1 subunit (α1R271Q/L) which cause the neuromotor disorder hyperekplexia are on example of such allosteric mutations. In this issue of the *BJP*, Shan *et al.* show that normal function was restored to these mutant receptors by substitution of the segment which contained the mutated position, by a homologous one. An allosteric drug could mimic the effects of such substitution. Within this framework, I highlight the advantages of allosteric drugs and the challenges in their design.

LINKED ARTICLE
This article is a commentary on Shan *et al.*, pp. 2113–2123 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01701.x

Abbreviations
Akt, protein kinase B; allo-network drugs, allosteric drugs which act via the cellular network;; α1R271Q/L, glycine receptor subunit α1 with Arg^{271} substituted by glutamine or lysine

Transmembrane receptors, such as those for amino acid neurotransmitters, which communicate between the extracellular environment and the cellular interior, can be stimulated by ions, small molecules, amino acid residues, hormones, neurotransmitters or covalent modification events. A signalling molecule binds to the extracellular domain to initiate a cascade of intracellular protein interaction events that eventually activate (or repress) a gene and, in this way, specify cellular expression. A key first step is the communication of the information of the signalling molecule binding from the extracellular domain to the intracellular domains. In the glycine receptor α1 subunit, the α1R271Q/L disease-related mutations which are distant from the glycine binding site, interrupt the allosteric communication between the extra- and intracellular domains, and in this way cause the neuromotor disorder, hyperekplexia. However, as shown in this issue of the *BJP*, Shan *et al.* (2012) restored function to the mutated receptor by substituting the protein segment which contains the α1R271Q/L mutations by a homologous segment. Further, the glycine receptors were functional even when other mutations were engineered at this position (Arg^{271}). These experimental results support the proposition that disease-related mutations may be harmful by causing changes in the allosteric communication pathways (del Sol

et al., 2009), and suggest that even a slight modulation of the microenvironment by a drug could be a therapeutic target to overcome the mutational effects.

Drugs which act as allosteric modulators are increasingly popular, as can be judged from any search of the literature or drug pipelines (Conn *et al.*, 2009; Kenakin and Miller, 2010). This is because of three main reasons: first, and most importantly, they are expected to have fewer unwanted side effects than orthosteric drugs; second, they allow modulation of drug therapy effects; and third, they mostly act only when the endogenous ligand is bound. The fewer side effects, or lower toxicity, derive from their greater specificity. Orthosteric drugs bind at the active sites, which tend to be more conserved across protein families than the remainder of the protein surface (Ma *et al.*, 2003; Capra *et al.*, 2009); thus, drugs that bind the active site of one protein may also bind those of homologous proteins. By contrast, allosteric drugs bind elsewhere on the protein surface and affect function via long-range communication. They allow modulation, because unlike orthosteric drugs which block the active site and shut off protein activity, they bind elsewhere; and because the communication pathways spread in the structure, the effect on the active site is partial, and in principle, tunable. And finally, because they are non-competitive, they normally exert their effect only when the natural ligand is bound. Because endogenous ligand binding is regulated by the cellular environment, they act when the biological state of the cell needs their action; that is, they preserve the natural cellular rhythm. To understand these effects and what can happen in the presence of a disease-causing mutation, we need to consider the origin of the communication pathways and how they spread (del Sol *et al.*, 2009). Proteins are flexible and information flows through dynamic changes in the distribution of their ensembles. The key to successful allosteric drug discovery is accounting for these dynamic changes. These changes reflect the cellular environment, such as concentrations of metabolites or ions, the presence of low MW transmitters, changes in pH and post-translational modification events, along with mutational events. Via protein–protein interactions, the dynamic changes in the protein ensemble propagate via the signalling pathways of the cell and affect the responses of the cell (Kar *et al.*, 2010).

How does allostery work, and how does the dynamic modulation take place? The surfaces of proteins are in contact with their environment: water molecules, membrane lipids, solutes, etc. An allosteric drug will displace some of these contacts and now instead of an optimal interaction between the protein surface atoms and, for example, water molecules, those surface atoms interact with the drug. This causes strain, because once surface atoms shift to optimize the interactions with drug atoms, they, and the residues they belong to, are not able to comfortably retain their conformations and interactions with their neighbours. To relax the strain, their positions need to change. However, in turn, these changed positions and interactions will now force their neighbours to change their contacts and conformations as well. In this way, the strain energy propagates throughout the structure of the protein. This is the origin of the allosteric effect and allosteric pathways. The strain energy propagates through multiple pathways; some of which are major, others are minor. Because all possible conformations of the protein pre-exist in the ensemble, propagation implies flipping between these conformers in the ensemble, that is, between 'conformational states'. In major pathways, the barriers between successive states are lower; in minor pathways, some of the barriers are higher. The barrier heights reflect the time it would take to propagate an induced change along the pathway between the perturbation site (the ligand binding site) and the second (substrate) site. Mutations may change the barrier heights and thus alter the relative occupancy of the states and pathways. Disease-causing mutations are frequently on major pathways. This may affect the extent of the functionally needed conformational change at the substrate binding site.

This appears to be the case for R271 in the glycine receptor α1 subunit, where the R271Q and R271L (α1R271Q/L) mutations cause the hereditary neuromotor disorder, hyperekplexia (Zhou *et al.*, 2002), where the glycine receptors have reduced ability to conduct chloride ions. The amino acid residue R271 is at the extracellular mouth of the channel pore, between the agonist binding sites and channel gate (Hibbs and Gouaux, 2011)and the α1R271Q/L mutations reduce sensitivity to the endogenous agonist, glycine (Lynch, 2004). From the standpoint of conformational dynamics, this reduced sensitivity could reflect a change in the allosteric propagation pathways, making a major pathway that, in the wild-type functional state, passes through the contacts of R271 into a minor one. Shan *et al.* (2012) show that by replacing a 12-residue homologous segment taken from the glycine receptor β subunit which includes R271, the function of the α1R271Q/L glycine receptors was restored. Further, the engineered protein was resistant to some other R271 substitutions, and the conformational change around this residue was uncoupled from the channel gating. Thus, the homologous segment, which contains some different amino acid residues, modified the free energy landscape, and redistributed the pathways, restoring agonist sensitivity.

In practice, i.e., *in vivo*, segment substitution is not a therapeutic option. Instead, in principle, a drug that mimics such effects could be designed. The challenge is how to find an appropriate allosteric site, and how to design an appropriate drug which will change the relative occupancy of the pathways such that function will be restored. This could be difficult for transmembrane receptors which play essential roles in multiple key signalling pathways; however, it could be much more challenging for globular proteins. Unlike an orthosteric drug which binds at the active site and blocks it, the location of the appropriate allosteric site is unclear, and the properties defining it are not entirely understood. Further, even if there are hints to the allosteric sites, defining which contact types and combinations between the drug and the protein will elicit the desired pathways constitute another major challenge. The problem is compounded by the challenge of the modulation of the drug effects which is sought. The results provided by Shan *et al.* (2012) take a step in this direction. Beyond the validation of the theoretical proposition (del Sol *et al.*, 2009), they illustrate the potential effectiveness of targeting the microenvironment of the mutated residue. The substitution of a segment (slightly) shifted the allosteric pathway and overcame the detrimental effect of the mutation while retaining the function of the native protein. While no statistics are currently available, it is reasonable to assume that, particularly for cases where the mutation is near

the endogenous ligand binding site, the microenvironment is a natural place to start. A drug which targets the pathway and slightly shifts and modulates it could be promising.

Despite their advantages, allosteric drugs also present problems and chief among these is acquired resistance. As in the case of orthosteric drugs, mutations can overcome the therapeutic effect, again by shifting the allosteric pathways. Allosteric drugs can induce activation rather than repression as in the case of the phosphorylation of PKC and Akt (Cameron *et al.*, 2009; Okuzumi *et al.*, 2009). In addition, the problem of the cell utilizing parallel pathways (Engelman and Settleman, 2008; Sequist *et al.*, 2011) is also a major hurdle (Corcoran *et al.*, 2010). Overcoming these could involve multi-drug regimes, with combinations involving both allosteric and orthosteric drugs. Among these, the newly proposed 'allo-network drugs', which act by binding to another protein in the signalling pathway and whose effects propagate through the cellular network across several proteins (Nussinov *et al.*, 2011), present a novel paradigm. Further, a key advantage of allosteric drugs is that the dosage can be low, because they are not competitive with the endogenous ligands and do not bind homologous proteins; however, their effects may be transient, which is also a disadvantage in a chronic or debilitating disease. Thus, the challenge is still immense; however, as inspection of the literature shows, there is much progress and increasing numbers of successes.

Acknowledgements

Work in my laboratory cited here has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health (NIH), under contract number HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government. This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Conflict of interest

I declare that there is no conflict of interest.

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