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COMMENTARY

The tail wags the dog: possible mechanism for reverse allosteric control of ligand-activated channels

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In this issue of the *British Journal of Pharmacology*, a new article by Kozuska *et al.* discusses the multiple salt bridges in the intracellular domain of the $5HT_{3A}$ receptor. These interactions increase the overall rigidity of the receptor, stabilize its low conducting state and affect the ligand cooperativity. The authors suggest that the allosteric effects of these regions on the receptor may be involved in a possible 'reverse' allosteric modulation of $5HT_3$ receptors.

LINKED ARTICLE
This article is a Commentary on Kozuska *et al.*, pp. 1617–1628 volume 171 issue 7. To view this paper visit http://dx.doi.org/10.1111/bph.12536

Abbreviations
pLGIC, pentameric ligand-gated ion channel

Thirty years separated the first attempts to characterize serotonin receptors in guinea pig ileum (Gaddum and Picarelli, 1957) and the discovery of the serotonin-activated channel known as the $5HT_{3A}$ receptor (Bradley *et al.*, 1986; receptor nomenclature follows Alexander *et al.*, 2013). Most serotonin receptors (~14 different subtypes) belong to the GPCR superfamily, but $5HT_3$ receptors belong to the pentameric ligand-gated ion channel (pLGIC) family. These serotonin receptors are cation channels rapidly activated by serotonin to generate inward desensitizing currents of sodium and potassium. These receptors are widely expressed, and their activation or inactivation affects vagal transmission of intestinal and cardiac stimuli, as well as other CNS-related effects on cognition, anxiety and nociception. $5HT_3$ receptor antagonists are used to block some of the nauseating effects generated by chemotherapeutic agents in cancer treatments, presumably by blocking their vagal effects (Farber *et al.*, 2004).

pLGICs are composed of five identical subunits (homomeric channels) or various combinations of several different subtypes (heteromeric channels). There are four main types of pLGICs: the cationic channels that respond to ACh and serotonin and the anionic channels that respond to GABA and glycine. The direct structural information we currently have regarding these channels comes from the electron microscopy-derived structure of the nicotinic ACh receptor (nAChR) from the electric organ of *Torpedo marmorata* (Unwin, 2005) and crystallization of the extracellular domains as well as complete receptors (although somewhat modified) from eukaryotic and prokaryotic sources (Nys *et al.*, 2013).

The different structures revealed a well-conserved architecture (Figure 1): pLGICs are allosterically regulated ion channels consisting of an extracellular ligand-binding domain, a transmembrane channel domain and an intracellular domain that affects channel conductivity (Nys *et al.*, 2013). All of the functional domains are formed in the interface between the subunits of the pentameric receptor (Nys *et al.*, 2013); thus, the ligand-binding site is located between the subunits in the extracellular domain, the channel is located in the interface formed by the second transmembrane domain (TM2) of the subunits, and the intracellular loops of each of the subunits form an intracellular domain whose effects on conductivity are now becoming clearer.

It was previously observed that the single-channel conductance of the heteromeric $5HT_{3AB}$ receptor is greatly enhanced relative to the homomeric receptor ($5HT_{3A}$). Mutation of arginine residues in the intracellular domain of the receptor resulted in a marked increase in the homomeric

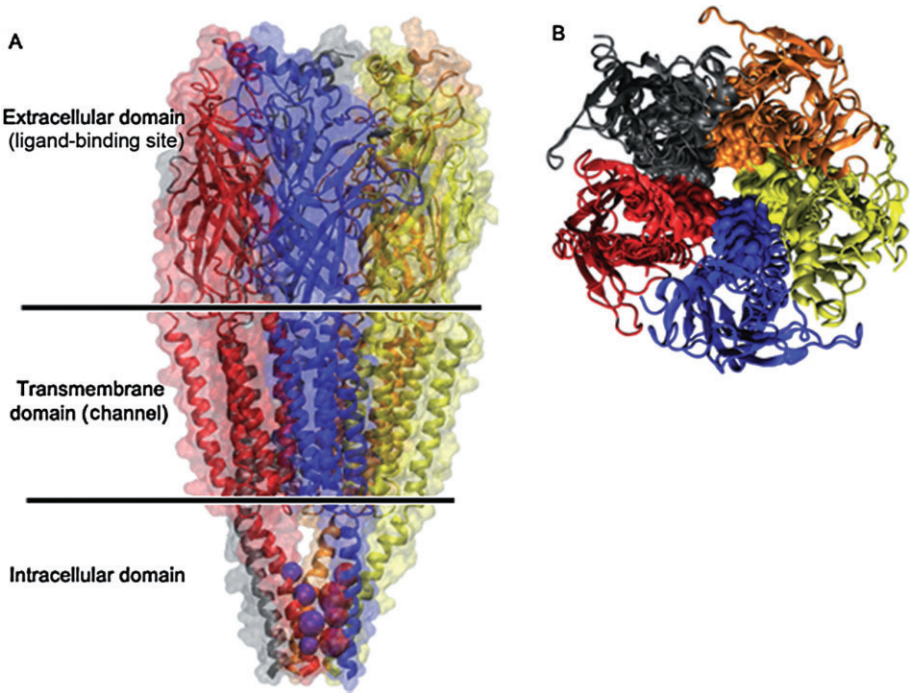

Figure 1

nAChR structure. Side view (A) and top view (B). The different subunits are in different colours. The ligand-binding site, in the extracellular domain, is in the interface between the subunits. The ion channel is in the interface between TM2 from all the subunits (represented as surface in B). The red and blue spheres in the intracellular domain represent residues in $5HT_{3A}$ that form putative salt bridges.

channel conductance. It was therefore suggested that the lower conductance of the homomeric channel is due to electrostatic and/or steric hindrance of cation passage through the arginine-lined portals in the intracellular domain of this pLGIC (Kelley *et al.*, 2003).

In their current paper, Kozuska *et al.* (2014) use a structural model of this region to study the molecular basis of the low conductance in the homomeric channel. The structural model, based on the structure of the nAChR, suggested the existence of salt bridges between positive and negative charges from different subunits in the structure, that increases the stability and rigidity of the channel (Figure 1). Based on the structural model, the authors introduced mutations into the portal region of the receptor that maintained the positively charged arginines, but that eliminated the putative salt bridges involving these arginine residues. The authors examined single channel conductance and whole-cell response to 5-HT in these mutants, and demonstrated substantial changes in channel gating and cooperativity that suggested increased flexibility throughout the protein.

From their results, it seems that the effects of the intracellular domain on channel conductance are not due to steric or electrostatic hindrance, but rather the overall stabilization of the low conducting state of the channel. The ligand-binding domain is an allosteric regulator of the channel, but the work of Kozuska *et al.* (2014)provides important evidence for the existence of a reverse allosteric communication in the receptor. The authors show that mutations in the intracellular domain can affect not only channel conductance but also ligand cooperativity, often thought of as the number of agonist molecules required to evoke maximal activation. The importance of the intracellular domain for this 'reverse allosteric conductance' may prove to be another level of control of these important channels. Intracellular proteins are known to interact with pLGIC proteins and there is evidence for their effects on signalling. These types of intracellular proteins may be able to interact with the intracellular domain of the channel and thus allosterically modify the protein properties.

Conflict of interest

None declared.

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