JGP Journal of General Physiology

COMMENTARY

Elucidating the clandestine behavior of enantiomeric DHPs in calcium channels

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One only need examine the recent history of the pharmaceutical industry to see the difficulties that can arise with enantiomeric molecules. For instance, thalidomide, originally marketed as a sedative, exhibits such a phenomenon, with *S*-thalidomide causing severe birth defects as opposed to the desired sedative effect of *R*-thalidomide. Due to these problematic issues, investigations into antithetical effects in enantiomers remains extremely relevant in contemporary pharmacology research. Such an effect is found in 1,4-dihydropyridine (DHP) Bay k 8644, an enantiomeric ligand which acts as both agonist (*S*) and antagonist (*R*) against the L-type calcium channels. There have been continuing efforts among researchers developing accurate pharmacological models for agonists and antagonists on these channels. The study by Tikhonov and Zhorov (2023) is dedicated to this topic using computational approaches.

The L-type calcium channels belong to the subgroup of voltage-gated calcium (Cav) channels, where "L" refers to its long-lasting activation duration. These channels are critical in the body, uniting membrane depolarization in neurons to processes such as gene expression, synaptic efficacy, hormone secretion, and cell survival (Lipscombe et al, 2004). Advancements in cryo-electron microscopy (cryo-EM) techniques have provided structures of apo and ligand-bound Cav channels (Zhao et al., 2019a, 2019b; Gao and Yan, 2021), enabling extensive in silico methods for determining specific binding poses and interactions within receptor–ligand complexes, giving an insight into the exact binding modes of DHP agonists and antagonists (Fig. 1 A). The study by Tikhonov and Zhorov (2023) has introduced a new hypothesis regarding the contradictory effects of the enantiomeric DHPs, which is related to a π-bulge within the S6 segment in the pseudo-tetrameric channel.

π-bulges or π-helices are a secondary structure in proteins characterized by having one extra residue per turn compared to the standard α-helix. What is interesting about π-helices is their rarity in resolved protein structures, accounting for ∼15% of entries in the RCSB database (Cooley et al., 2010). This rarity is due to an energetically unfavorable central helical hole, indicating that such a deformity is only present for some evolutionary beneficial justification (Riek and Graham, 2011). Additionally, the occurrence of π-helices is often correlated with the binding site of proteins, either by formation or stabilization of the site (Weaver, 2000). In recent structures of sodium, calcium, and TRP channels, π-bulges have been frequently observed, raising questions about their functional implications. The study by Tikhonov and Zhorov (2023) suggest that these π-bulges play an integral role in the gating mechanisms of Cav and Nav channels, governing the binding of *R*/*S* Bay k 8644 (Fig. 1 B).

The original cryo-EM structures bound with DHP agonists and antagonists revealed the same Cav conformation, presumably representing the inactivated state. Drs. Tikhonov and Zhorov used a series of cryo-EM structures of the eukaryotic sodium and calcium channels, both with and without π-bulges in segments $S6_{III}$ and $S6_{IV}$, as structural templates in their in silico investigation. By using molecular docking complemented by Monte Carlo (MC) energy minimization, the authors ascertained the differences between the two enantiomers. The molecule itself, *R*/*S* Bay k 8644 is a penta-substituted DHP derivative with methoxycarbonyl, 2-trifluoromethyl phenyl, and nitro substitutions at positions 3, 4, and 5, respectively (Fig. 1 C). There are two methyl substituents at positions 2 and 6, with the asymmetric center located at the fourth position. The energy minimizations of the initial structures with only the α-helices revealed the difference in energy profiles between the enantiomers. The *R*-form exhibited the most favorable profile, with the methoxy group fitting smoothly into the binding pocket. Conversely, in the *S*-form, this methoxy group is replaced by the NO_2 group, which when bound to a water molecule causes destabilization of the binding pocket due to the repulsion from the pocket, resulting in an energy difference of +1.8 kcal/mol.

The most interesting results from the authors were upon the inclusion of the π-bulges in the key helices $S6_{III}$ and $S6_{IV}$. What was found was that, due to the deformations of the helices, two key residues were reorientated to provide favorable interaction conditions for the enantiomer, depending on its absolute configuration. The residue Ile^{4i19} was orientated away from the binding pocket, replaced by an Asn^{4i20}. This Asn residue possesses a polar side chain, altering the binding pocket environment to become

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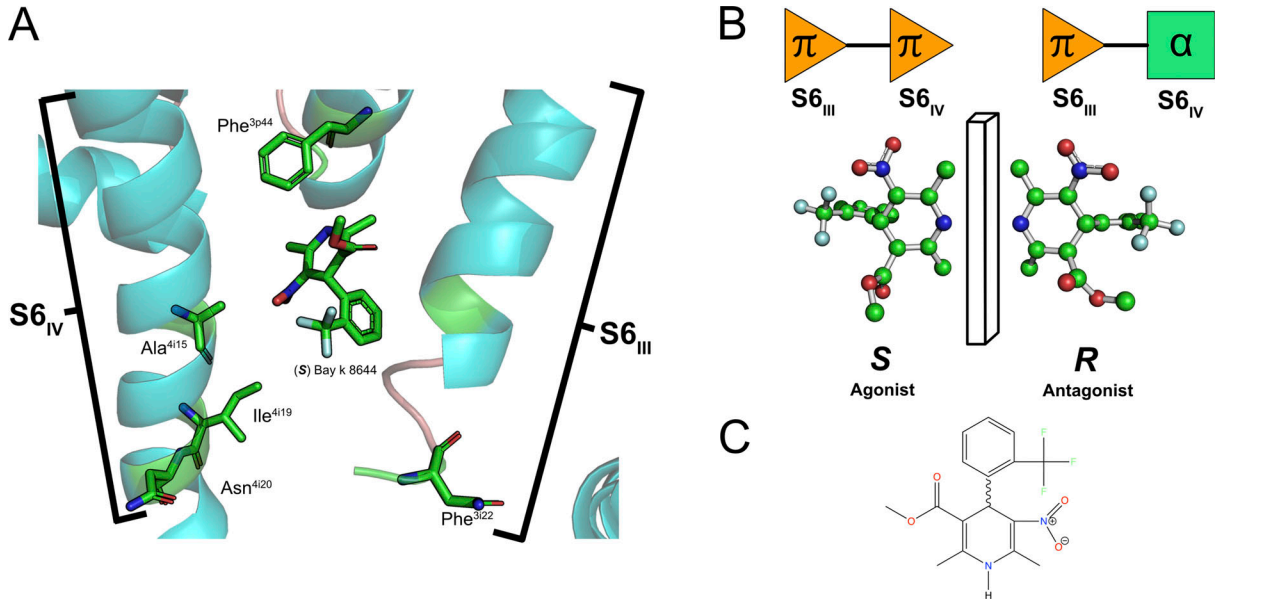

Figure 1. **Binding pocket and modulation mechanism of Bay k 8644 in Cav1.1 channel. (A)** Close-up of the binding pocket of Cav1.1 with *S* Bay k 8644, taken from the cryo-EM structure (PDB: 7JPL) (Gao and Yan, 2021). **(B)** Depiction of the two enantiomers of Bay k 8644 and their preferred helical conformation in $S6_{III}$ and $S6_{IV}$ shown above. The authors found that the *S* enantiomer, the agonist, had more favorable interactions with the Cav channel when both the $S6_{III}$ and $S6_{IV}$ helices were π-helices. The *R* enantiomer, the antagonist, was found to have more favorable interactions when the $S6_{III}$ helix was a π-helix, and the $S6_{IV}$ an α-helix. **(C)** Molecular graph of Bay k 8644, with the chiral center indicated.

more accommodating to the *S*-form, when the nitro group is bound with a water molecule. Therefore, in models containing the π-bulged $S6_{IV}$ helix, the *S*-form was found to be more attractive as the water molecule was now at the optimum distance. Among all models analyzed, those with fully α-helical $S6_{III/IV}$ were unfavorable to both DHP agonist (*S*) and antagonist (*R*), models with α-helical $S6_{IV}$ and π-bulged $S6_{III}$ favorable only to the antagonist (*R*), and models with π-bulged $S6_{III}$ and S_{IV} favorable to the agonist (*S*) when water is bound to the nitro group (Fig. 1 B).

What Drs. Tikhonov and Zhorov have uncovered is a glimpse into the complexities surrounding the mechanisms that govern the interactions for DHPs in calcium channels. Additionally, they have begun to demystify the clandestine functions of π-helices often found in close proximity to the active site of proteins. In this case study, the presence of the π-deformation is key to determining the preference of ligand binding to this enantiomeric compound. The reorientation of residues enables more preferable binding for DHPs, bringing the stabilising Asn^{4i20} towards the binding pocket. The suggestion, therefore, is that Cav channels with the π-bulged $S6_{III}$ and α-helical $S6_{IV}$ represent an inactivated state, and those with both $S6_{III}$ and $S6_{IV}$ π-bulged represent the open state. This hypothesis aligns with recent studies by Delemotte et al., where molecular dynamics simulations suggested that the conformation of π-bulging, present in both $S6_{III}$ and $S6_{IV}$ of Nav channels, is the activated open structure (Choudhury et al., 2022; Choudhury and Delemotte, 2023). The hypothesis of several residues required for DHP binding, and the involvement of π-bulges in these mechanisms, provides a welcome insight into the understanding of the complexities of the gating in L-type calcium channels. This, in turn, will inspire other structural and computational biologists to investigate this mechanism from various perspectives.

Acknowledgments

Eduardo Ríos served as editor.

This work was funded by the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP).

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