PUTATIVE MOLECULAR MECHANISM OF BHQ-MEDIATED MODULATION OF CAV2.1 CHANNELS: INSIGHTS FROM FUNCTIONAL STUDIES AND RECENT CRYO-EM STRUCTURES

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Abstract - 2, 5-Di-tert-butylhydroquinone (BHQ), originally identified as a SERCA inhibitor, also directly modulates the gating of the P/Q-type calcium channel CaV2.1. BHQ suppresses voltage-dependent activation, slows deactivation, and enhances Ca^{2+}-dependent facilitation, indicating an allosteric effect on channel gating rather than pore blockade. Based on recent cryo-EM structures of human CaV2.1, the hydrophobic cavity at the interface between the voltage-sensing domain and pore domain, particularly around the S4–S5 linker, represents the most plausible BHQ-binding site. Binding at this interface may alter electromechanical coupling, providing a unified mechanism for the observed changes in activation, deactivation, and Ca^{2+}-dependent facilitation. Future structural and functional studies are required to validate this proposed mechanism.

Keywords - CaV2.1, 2, 5-Di-tert-Butyl Hydroquinone (BHQ), Gating Modulation, Cryo-EM Structure

I. INTRODUCTION

2,5-Di-tert-butylhydroquinone (BHQ) has traditionally been regarded as a pharmacological inhibitor of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) and has therefore been widely used as an experimental tool for depletion of intracellular Ca^{2+} stores. Inhibition of SERCA decreases Ca^{2+} sequestration into the endoplasmic reticulum, leading to gradual depletion of intracellular Ca^{2+} stores and activation of store-operated calcium entry. Consequently, BHQ has been extensively employed in studies of intracellular Ca^{2+} signaling, excitation–contraction coupling, endoplasmic reticulum stress, and intracellular calcium homeostasis (1,9). However, accumulating electrophysiological evidence has demonstrated that BHQ exerts an additional action that is mechanistically distinct from SERCA inhibition. Rather than acting solely through depletion of intracellular Ca^{2+} stores, BHQ directly modulates the gating behavior of the P/Q-type voltage-gated calcium channel CaV2.1 (2). This finding substantially broadens the pharmacological significance of BHQ and suggests that previous studies employing BHQ as a selective SERCA inhibitor should be interpreted cautiously, particularly in neurons and other excitable cells in which CaV2.1 contributes significantly to calcium influx.

The study by Inagaki and colleagues provided the first compelling evidence that BHQ directly modifies CaV2.1 channel function independently of intracellular Ca^{2+} store depletion (2). BHQ suppresses voltage-dependent activation, slows channel deactivation, enhances Ca^{2+} -dependent facilitation (CDF), and partially rescues the abnormal gating phenotype of the familial hemiplegic migraine type 1 (FHM1)-associated S218L mutant. Importantly, these effects were not reproduced by structurally unrelated SERCA inhibitors, including thapsigargin and cyclopiazonic acid, indicating that depletion of intracellular Ca^{2+} stores alone cannot explain the observed electrophysiological changes (2). Instead, the results strongly support the conclusion that BHQ interacts directly with the CaV2.1 channel complex or with structural elements intimately involved in channel gating. This distinction is pharmacologically significant because it identifies BHQ as one of the few small molecules capable of modifying CaV2.1 gating through an allosteric mechanism rather than by direct pore occlusion.

The electrophysiological profile produced by BHQ differs fundamentally from that of conventional calcium channel blockers. Classical pore-blocking compounds generally reduce macroscopic current amplitude by physically obstructing ion permeation or by stabilizing nonconducting channel conformations. In contrast, BHQ produces only a modest reduction in peak current while inducing a pronounced depolarizing shift in the voltage dependence of activation together with significant slowing of channel deactivation (2). These observations indicate that BHQ primarily alters the energetic landscape governing channel activation rather than directly interfering with ion permeation. Such behavior is characteristic of an allosteric gating modifier, in which ligand binding changes the equilibrium among resting, activated, and open conformational states without physically occluding the ion-conducting pathway (3,4).

This interpretation is highly consistent with the established mechanism of electromechanical coupling in voltage-gated calcium channels. Voltage-dependent activation begins with outward displacement of the positively charged S4 helices located within each voltage-sensing domain in response to membrane depolarization. Mechanical movement of these voltage sensors is transmitted through the intracellular S4–S5 linker to the S6 helices, which together form the

activation gate of the pore (3,4). Structural and electrophysiological studies have demonstrated that even subtle perturbations within this coupling interface can simultaneously alter activation voltage dependence, activation kinetics, deactivation kinetics, and channel open probability. Consequently, a ligand binding within this region would be expected to influence multiple gating parameters while producing only limited effects on maximal ionic conductance. The unique electrophysiological phenotype produced by BHQ is therefore remarkably consistent with modulation of the electromechanical coupling machinery rather than direct interaction with the ion-conducting pore itself (2–4).

Another characteristic feature of BHQ is its marked slowing of channel deactivation. Deactivation represents the reverse transition from the activated open state to the resting closed state following membrane repolarization. This process requires coordinated relaxation of the voltage-sensing domains and subsequent closure of the intracellular S6 activation gate through the electromechanical coupling machinery. BHQ significantly prolongs deactivation kinetics, suggesting stabilization of activated conformations or an increase in the energetic barrier separating activated and resting states (2,4). At first glance, the coexistence of slower deactivation and a depolarizing shift in activation may appear paradoxical. However, both effects can be explained by alterations in the same allosteric energy landscape. Rather than selectively promoting either channel opening or closure, BHQ may reduce the conformational flexibility of the voltage sensor–pore domain interface, thereby slowing structural transitions in both directions. Similar alterations in activation and deactivation kinetics have been observed for mutations affecting the S4–S5 linker and neighboring S6 helices, further supporting the hypothesis that BHQ acts primarily on the electromechanical coupling machinery rather than on the permeation pathway itself (3,4).

An equally intriguing aspect of BHQ pharmacology is its enhancement of Ca^{2+} -dependent facilitation (CDF), one of the defining regulatory properties of CaV2.1 channels. CDF plays an essential role in short-term synaptic plasticity by increasing calcium influx during repetitive neuronal activity. This process is mediated by calmodulin (CaM), which remains constitutively associated with the C-terminal IQ domain of the CaV2.1 α1A subunit (5). Upon binding Ca^{2+} , CaM undergoes conformational rearrangements that promote subsequent channel opening while coordinating the balance between facilitation and Ca^{2+} -dependent inactivation (CDI) (5,6). Although BHQ markedly enhances CDF, no biochemical, structural, or electrophysiological evidence currently demonstrates direct interaction between BHQ and CaM or with the IQ domain. Consequently, the available evidence favors an indirect mechanism whereby BHQ-induced conformational changes within the transmembrane region are transmitted allosterically to intracellular regulatory domains. Such long-range communication between transmembrane gating machinery and cytoplasmic regulatory elements has become a well-recognized principle in voltage-gated ion channel biology and provides a plausible explanation for enhancement of CDF without direct modulation of CaM itself (3,5,6).

The ability of BHQ to partially normalize the gating defects produced by the S218L mutation provides additional evidence supporting this interpretation. Familial hemiplegic migraine type 1 (FHM1) results from gain-of-function mutations in the **CACNA1A** gene encoding the CaV2.1 α1A subunit. These mutations generally shift channel activation toward more hyperpolarized membrane potentials, increase channel open probability, and enhance presynaptic Ca^{2+} entry, thereby facilitating glutamate release and increasing susceptibility to cortical spreading depolarization (7). Among these variants, S218L is one of the most severe mutations and is associated with early disease onset, cerebellar dysfunction, seizures, and prolonged migraine attacks (7). Remarkably, BHQ partially restores normal voltage-dependent gating of the S218L mutant by shifting activation toward more depolarized potentials and reducing the gain-of-function phenotype (2). This pharmacological correction indicates that BHQ does not simply suppress channel activity but instead rebalances the energetic relationship between resting and activated conformations. Such behavior distinguishes BHQ from conventional calcium channel inhibitors and raises the possibility that selective allosteric modulators could be developed to normalize pathological gating in CaV2.1 channelopathies without completely abolishing physiological calcium influx.

II. INSIGHTS FROM RECENT CRYO-EM STUDIES

Recent advances in cryo-electron microscopy have substantially transformed our understanding of CaV2.1 channel architecture. High-resolution structures of human CaV2.1 have revealed the detailed organization of the four voltage-sensing domains, the central pore domain, intracellular β subunit, extracellular α2δ subunit, and multiple membrane-facing cavities surrounding the transmembrane helices (8). These structures have also clarified the structural basis of subtype-specific toxin recognition and have provided unprecedented insight into the dynamic coupling between voltage sensing and pore opening. Importantly, they now offer a robust structural framework for interpreting the unique electrophysiological effects of BHQ and for developing experimentally testable hypotheses regarding its molecular binding site.

Although no experimental structure of BHQ bound to CaV2.1 has yet been reported, integration of electrophysiological observations with recently determined cryo-electron microscopy structures allows several mechanistic hypotheses to be proposed. Among the candidate binding sites, the interface between the voltage-sensing domain (VSD) and the pore domain (PD) surrounding the S4–S5 linker appears to represent the most plausible region for BHQ interaction (3,4,8). This interface forms a membrane-accessible hydrophobic cavity that is sufficiently large to accommodate relatively small lipophilic aromatic compounds. BHQ contains two bulky tert-butyl substituents that are expected to partition readily into the lipid bilayer and interact favorably with hydrophobic residues lining this cavity. Simultaneously, its two hydroxyl groups may establish hydrogen-bond interactions with nearby polar amino acids, thereby stabilizing a preferred conformational state of the channel. Occupancy of this interface could alter force transmission from the voltage-sensing domains to the intracellular S6 activation gate, providing a unified structural explanation for the experimentally observed suppression of activation, slowing of deactivation, enhancement of Ca^{2+} -dependent facilitation, and partial correction of FHM1-associated gating abnormalities (2–4,8).

Nevertheless, alternative binding sites should not be excluded. Membrane fenestrations connecting the lipid bilayer with the central cavity have emerged as important access pathways for hydrophobic ligands in several voltage-gated sodium and calcium channels. Because BHQ is considerably more hydrophobic than hydroquinone itself, lateral diffusion through these fenestrations remains mechanistically feasible (4,8). Likewise, the extracellular surface of voltage-sensing domain IV contributes to the binding of peptide gating modifiers such as ω-agatoxin IVA and forms part of a broader allosteric network regulating voltage-sensor movement (8). Although BHQ is structurally unrelated to peptide toxins, interaction with neighboring membrane-exposed surfaces cannot presently be excluded. However, the collective electrophysiological evidence favors modulation of the VSD–PD interface rather than direct pore blockade or interaction with intracellular calmodulin-binding domains, because the functional phenotype produced by BHQ is dominated by alterations in gating kinetics rather than by marked suppression of ionic conductance (2–4).

III. FUTURE DIRECTIONS

The structural hypothesis proposed here remains speculative and requires rigorous experimental verification. Molecular docking studies using the latest high-resolution CaV2.1 structures should first identify energetically favorable BHQ-binding pockets. Subsequently, long-timescale molecular dynamics simulations could evaluate the stability of ligand binding and determine how BHQ influences voltage-sensor movement, S4–S5 linker flexibility, and pore opening. These computational approaches should be complemented by systematic site-directed mutagenesis of residues predicted to line the proposed binding cavity, followed by electrophysiological analysis to determine whether mutation of individual amino acids attenuates BHQ sensitivity. Such an integrated structural–functional strategy would provide direct experimental evidence for the molecular determinants of BHQ action and substantially strengthen the proposed mechanistic model (4,8).

Beyond elucidating the mechanism of BHQ itself, identification of its binding site may have broader implications for ion-channel pharmacology. Most currently available CaV2.1 inhibitors either lack subtype selectivity or suppress calcium influx indiscriminately, thereby limiting their therapeutic utility because of adverse effects on normal synaptic transmission (3). In contrast, compounds that selectively modify electromechanical coupling without complete pore blockade may normalize pathological gating while preserving much of the physiological function of the channel. This concept is particularly attractive for neurological disorders caused by gain-of-function mutations, including familial hemiplegic migraine type 1, in which restoration of normal gating is likely to be more beneficial than complete inhibition of channel activity (2,7).

Furthermore, the present hypothesis raises the possibility that BHQ may serve as a prototype scaffold for the development of a new class of allosteric CaV2.1 modulators. Structure-guided optimization of the hydroquinone core, modification of hydrophobic substituents, and rational adjustment of hydrogen-bonding capacity may improve subtype selectivity, binding affinity, and pharmacokinetic properties. Such medicinal chemistry efforts, combined with advances in cryo-electron microscopy, artificial intelligence-assisted structural prediction, and molecular simulation, may facilitate the rational design of precision therapeutics targeting CaV2.1 gating.

In conclusion, integration of electrophysiological evidence with recently determined cryo-electron microscopy structures supports a model in which BHQ functions as an allosteric gating modifier rather than a classical pore-blocking inhibitor. Among the candidate structural regions, the hydrophobic VSD–PD interface surrounding the S4–S5 linker provides the most coherent explanation for the characteristic electrophysiological effects of BHQ, including suppression of activation, slowing of deactivation, enhancement of Ca^{2+}-dependent facilitation, and partial correction of FHM1-associated gating defects. Although this model remains hypothetical, it provides a clear conceptual framework for future structural, computational, and electrophysiological

investigations. Experimental identification of the BHQ-binding pocket will not only clarify the molecular pharmacology of this widely used SERCA inhibitor but may also establish a foundation for the rational development of next-generation allosteric modulators for CaV2.1-associated neurological disorders.

REFERENCES

[1] Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPase family of calcium pumps. J Biol Chem. 1991;266(26):17067–17071..

[2] Inagaki A, Frank CA, Usachev YM, Benveniste M, Lee A. Pharmacological correction of gating defects in the voltage-gated CaV2.1 Ca^{2+} channel due to a familial hemiplegic migraine mutation. Neuron. 2014;81(1):91–102..

[3] Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev. 2015;67(4):821–870.

[4] Catterall WA. Voltage-gated calcium channels. Cold Spring Harb Perspect Biol. 2011;3(8):a003947

[5] Lee A, Wong ST, Gallagher D, Li B, Storm DR, Scheuer T, Catterall WA. Ca^{2+}/calmodulin binds to and modulates P/Q-type calcium channels. Nature. 1999;399(6732):155–159.

[6] Chaudhuri D, Issa JB, Yue DT. Elementary mechanisms producing facilitation of Cav2.1 channels. J Gen Physiol. 2007;129(5):385–401.

[7] Pietrobon D. CaV2.1 channelopathies. Pflugers Arch. 2010;460(2):375–393.

[8] Li Z, Cong Y, Wu T, et al. Structural basis for different ω-agatoxin IVA sensitivities of the P-type and Q-type CaV2.1 channels. Cell Res. 2024;34(6):455–469.

[9] Toyoshima C, Nomura H. Structural changes in the calcium pump accompanying the dissociation of calcium. Nature. 2002;418(6898):605–611.

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