## RESEARCH HIGHLIGHT A tale of two calcium channels: structural pharmacology of Cav2.1 and Cav3.2

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Voltage-gated calcium channels control a plethora of physiological and pathophysiological processes and are important targets for therapeutic intervention into conditions such as epilepsy, pain, and cardiovascular diseases. Two elegant studies published in *Cell Research* provide structural insights into the molecular pharmacology of P/Q-type and T-type calcium channels.

The mammalian genome encodes ten different voltagedependent calcium channel isoforms that can be divided, based on their biophysical properties, into high and low voltage-activated channels, the former including the L-type (Cav1), P/Q-type (Cav2.1), N-type (Cav2.2) and R-type (Cav2.3) channels, and the latter encompassing the Cav3 T-type calcium channel family.<sup>1</sup> These calcium channel subtypes play distinct but sometimes overlapping physiological roles in skeletal and cardiac muscle contraction, synaptic transmission, calcium-dependent gene transcription and the regulation of neuronal excitability.<sup>1</sup> Conversely, calcium channel dysfunction has been associated with a host of cardiovascular and neurological conditions, including pain, migraine, ataxia, autism and cardiac arrythmias.1 Consequently, pharmacological inhibitors of calcium channels are used or being explored as possible therapeutics for these conditions.<sup>2</sup> The development of new pharmacological agents that target voltage-gated calcium channels is facilitated by a detailed understanding of the molecular structures of these channels, as exemplified by recently published cryo-EM structures of several members of the calcium channel family.<sup>3–5</sup> In two recent studies published in *Cell Research*, Li et al.<sup>6</sup> and Huang et al.<sup>7</sup> expand the current palette of calcium channel structures to two particularly important members — Cav2.1 and Cav3.2 (Fig. 1).

P- and Q-type calcium channels are important for neurotransmitter release in the central and peripheral nervous system, and have been associated with conditions such as familial migraine and ataxia.<sup>1</sup> They are formed by different alternatively spliced variants of the Cav2.1 subunit encoded by the *Cacna1a* gene, and differ in two important aspects. First, P-type calcium channels inactivate with much slower kinetics and this can likely be attributed to different association with auxiliary subunits. Second, P-type channels show a much greater sensitivity to inhibition by  $\omega$ -agatoxin IVA that is isolated from the venom of an American funnel web spider. A seminal study had previously revealed that an insertion of an asparagine and a proline residue into the fourth voltage sensing domain of Cav2.1 due to alternative splicing is a key determinant of  $\omega$ -agatoxin IVA sensitivity.<sup>8</sup> The new cryo-EM structure of Cav2.1 in complex with  $\omega$ -agatoxin IVA presented by Li et al.<sup>6</sup> confirms a key role of this region in regulating interaction between the channel and the toxin (Fig. 1a). Moreover, it is worth noting that  $\omega$ -agatoxin IVA is what is referred to as a "gating modifier" toxin because it acts by preventing the activation of the channel by voltage rather than blocking the pore.<sup>9</sup> The cryo-EM data are also consistent with a mechanism by which the toxin may prevent the outward movement of the voltage sensor region, thus locking the channel in a closed state. By contrast, the authors also created a cryo-EM structure of the channel bound to  $\omega$ -conotoxin MVIIC, a peptide isolated from the venom of the fish-hunting mollusk Conus magus. Unlike ω-agatoxin IVA, MVIIC is lodged within the permeation pathway of the channel, thereby occluding the pore and preventing calcium ions from passing through the channel (Fig. 1a). These structural data provide unparalleled new insights into the channel subtype specificity of different pore-blocking conotoxins for N-type and P/Q-type calcium channels. With the availability of a Cav2.1 structure, the stage is now set to use this information towards the rational design of new small organic inhibitors of these channels which may have therapeutic applications in conditions such as familial hemiplegic migraine, and which are currently lacking.

While Li et al.<sup>6</sup> focused on the Cav2.1 channel, Huang et al.<sup>7</sup> successfully solved the structure of Cav3.2 — the last remaining T-type calcium channel that had so far eluded resolution. This channel is particularly important because mutations in the gene encoding this channel have been linked to absence seizures, amyotrophic lateral sclerosis, and pain in humans.<sup>10</sup> The authors produced cryo-EM structural data of the channel in complex with four different types of small organic inhibitors — ACT-709478, TTA-A2, TTA-P2 and ML218 (Fig. 1b). These blockers belong to different classes of molecules, and all inhibit Cav3 channels in a non-selective manner. The authors present compelling evidence for two distinct modes of inhibition of calcium permeation through the pore. ACT-709478 and TTA-A2 both directly occlude ion flow by occupying the central cavity with their cyclic ring structures while their hydrophobic tails occupy the interface between domains I and IV of the channel. In contrast, the other two compounds lodge their cyclic groups into the fenestration between domains II and III and occlude ion permeation by extending their tails into the ion pathway. The authors elegantly identified individual contact points between blockers and the channels, and then used site-directed mutagenesis to confirm that replacement of key amino acid residues that form these contacts leads to altered blocking affinity in functional studies.

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Fig. 1 Antagonist interaction sites in P/Q-type Cav2.1 and T-type Cav3.2 channels. a Schematic diagram of a Cav2.1P/Q-type channel. Left, top view of the  $\alpha$ 1 subunit of Cav channels formed of four repeat domains (I–IV) each containing a voltage sensing domain (VSD<sub>I–IV</sub>, blue) and a pore module (PM<sub>I-IV</sub>, orange). For the sake of clarity, the auxiliary subunits Cav- $\alpha$ 2- $\delta$  and Cav- $\beta$  have been omitted.  $\omega$ -agatoxin IVA (AgaTx, green) binds residues in VSDIV of P-type Cav2.1 channel that alters the movement of the voltage sensor, affecting the activation of the channel. In Q-type Cav2.1 channels, additional residues in VSD<sub>IV</sub> modify the receptor site for AgaTx which decreases its inhibitory effect on the ionic current. w-conotoxin MVIIC (MVIIC, gray) binds residues in the pore modules preventing calcium ions to pass through the channel. Right, side view of the transmembrane topology of domain IV and domain II of the P/Q-type Cav2.1 channel. Numbers 1-6: transmembrane segments of domain IV. b Schematic diagram of the top view of a T-type Cav3.2 channel (middle). The chemical structures of ACT-709478 (ACT) and TTA-A2 (TA) reveal a similar cyclopropylphenyl head and aromatic tail arrangement (left). ACT and TA bind residues in the pore module of the channel: the head group interacts with residues in the central cavity, and the tail group makes contact with residues at the interface between domains I and IV. The structure of TTA-P2 (TP) and ML218 (ML) also reveals a similar arrangement with a 3,5dichlorobenzamide head and an aliphatic tail (right). TP and ML also bind residues in the pore module of the channel: the heads adhering to the interface between domains II and III and the tails projecting into the central cavity.

Importantly, the authors compared their findings on Cav3.2 with their previously published data on block of Cav3.1 channels by Z944, a compound structurally related to TTA-P2, revealing that the blocking site on Cav3.1<sup>4</sup> shares similarities with the TTA-P2/ML218 interaction site on Cav3.2. With this new structural information, it should now be possible to devise Cav3 isoform-specific inhibitors which is something that has so far eluded the field. In addition to these two antagonist sites, we note that Cav3 channels are also blocked by kurtoxin, a peptide isolated from the scorpion Parabuthus transvaalicus. Like w-agatoxin IVA, kurtoxin is an activation gating modifier,<sup>1</sup> thus suggesting the existence of at least a third antagonist site on Cav3 channels.

Besides the potential importance of these two studies for drug development, it is worth reflecting on the evolution of our understanding of ion channel structure. As many as five decades ago, long before the molecular cloning of ion channels, clever experimentation and reasoning correctly identified the molecular basis of ion channel inactivation.<sup>11</sup> Subsequently, biochemical measurements combined with photo-affinity tagging revealed L-type channel interaction domains for small organic molecules.<sup>1</sup> Further insights into channel function and their inhibition by drugs were gained by site-directed mutagenesis studies (such as those identifying the  $\omega$ -agatoxin IVA interaction determinants on P/Q-type channels) that are now being confirmed with exquisite details by cryo-EM structural work such as that presented by Li and colleagues.<sup>6</sup> We are thus in a new era of understanding ion channel function and their modulation by drug molecules that may pave the way to new therapeutics.

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## ADDITIONAL INFORMATION

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