COMMENTARY

How an intrinsic ligand tunes the activity of a potassium channel

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The KCNH family of ion channels (comprising the ether-à-go-go [EAG], EAG-related [ERG], and EAG-like [ELK] subgroups) are voltage-gated potassium channels that play important roles in regulating electrical activity in the brain and heart (Warmke and Ganetzky, 1994). These large proteins span the cell membrane, and their activation by membrane depolarization re- or hyperpolarizes the cell through potassium efflux. In this issue, Dai et al. investigate the role of an intrinsic ligand at the interface of two intracellular domains that define KCNH channels: the amino-terminal eag domain and the carboxy-terminal cyclic nucleotide-binding homology domain (CNBHD).

Several medical conditions have been linked to defects in the function of KCNH channels. Mutations in the human ERG (hERG) channel, for example, can result in long QT syndrome type 2 (LQT2), wherein the heart beats with an irregular rhythm because of improper repolarization of the action potential in cardiac cells (Sanguinetti and Tristani-Firouzi, 2006). Moreover, hEAG channels have been found to be overexpressed in several types of cancer (Pardo and Stühmer, 2014). Interestingly, several mutations associated with cancer and LQT2 have been mapped to the interface between two large intracellular domains that are only found in KCNH channels: the N-terminal eag domain and the CNBHD (Haitin et al., 2013).

A distinguishing feature of the eag domain–CNBHD interface is an intrinsic ligand formed by three amino acid side chains toward the C-terminal end of the CNBHD, which confers unique functional properties on KCNH channels (Brelidze et al., 2012; Marques-Carvalho et al., 2012; Haitin et al., 2013). The CNBHD does not bind cyclic nucleotides like the homologous cyclic nucleotide-binding domains in the related CNG and hyperpolarization-activated CNG (HCN) channels (Brelidze et al., 2009). Rather, the intrinsic ligand sits in the analogous cyclic nucleotide-binding pocket within the CNBHD and mimics the shape and orientation of a bound cyclic nucleotide (Brelidze et al., 2012; Marques-Carvalho et al., 2012). It appears that the intrinsic ligand could have evolved as a self-regulatory mechanism

to mimic the regulatory functions and/or conformational rearrangements associated with cyclic nucleotide binding and thereby ultimately regulate the gating process in KCNH channels. Indeed, recent studies have shown that mutations or deletions of the intrinsic ligand in KCNH channels can have complex effects on the gating properties of the channel (Brelidze et al., 2013; Zhao et al., 2017).

Dai et al. (2018) tackle the role of the intrinsic ligand in great detail. They provide a plausible mechanism for how the intrinsic ligand regulates KCNH channel gating by using an impressive combination of electrophysiological, biochemical, and fluorescence approaches, paired with mutations and deletions of the intrinsic ligand and/or components of the eag domain. Using these complementary approaches, Dai et al. (2018) study the dynamic regulation of a zebrafish ELK (zELK) channel by its intrinsic ligand and provide an elegant answer to previously unanswered questions about the involvement of the intrinsic ligand in the channel gating process.

Detailed insight into the regulatory role of the intrinsic ligand

Dai et al. (2018) study the role of the intrinsic ligand in voltage-dependent potentiation (VDP), a phenomenon by which depolarizing voltages slowly shift channels into a potentiated mode in which the open state of the pore is stabilized (Fig. 1; Dai and Zagotta, 2017). Deletion of the intrinsic ligand results in a loss of VDP, suggesting that the transition of the channel to a potentiated state with a stabilized open pore requires the intrinsic ligand. Furthermore, the intrinsic ligand is proposed (through the effects of its deletion) to be acting as an inhibitor in the resting state and an activator in the potentiated state, suggesting that the intrinsic ligand could undergo a slow conformational change that is eventually transferred to the gating machinery.

This notion is consistent with previous results showing that different mutations of two side chains of the intrinsic ligand that occupy the binding pocket result in modified gating properties to varying degrees (Zhao et al., 2017). Additionally, recent

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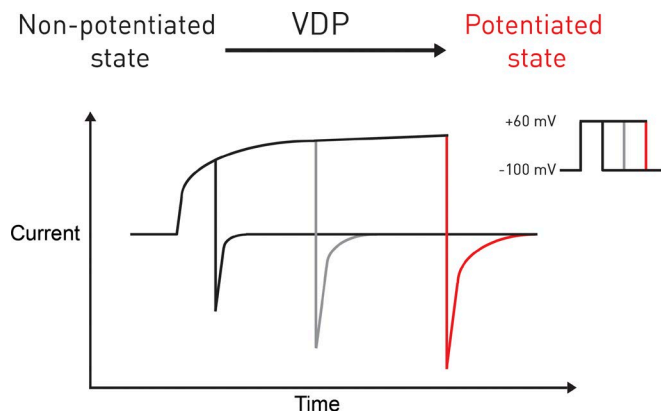Correspondence to Stephan A. Pless: stephan.pless@sund.ku.dk.

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Figure 1. **Illustration of VDP.** Trace represents increasingly potentiated currents elicited by a voltage protocol with increasing durations of a depolarizing pulse.

cryo–electron microscopy structures of related HCN channels in both cyclic nucleotide-bound and nucleotide-free forms suggest that cyclic nucleotide binding in the cyclic nucleotide-binding domain pocket induces a local conformational change, which is propagated to the pore via the counterclockwise rotation of the intracellular gating ring formed by the tetrameric assembly of C linkers just below the pore (Lee and MacKinnon, 2017). This raises the possibility that, although inherently tethered to its binding partner, the intrinsic ligand of the CNBHD in KCNH channels could be flexible enough to either adopt different conformations in the binding pocket or be totally/partially displaced from the pocket to mimic a ligand-bound or ligand-free cyclic nucleotide-binding domain. To directly test this hypothesis, Dai et al. apply exogenous peptides of the intrinsic-ligand sequence to channels lacking the intrinsic ligand. Their results show that the peptide can both bind to and regulate the channel in a similar way as the intrinsic ligand (acting as an antagonist in the resting state and an agonist in the potentiated state). This indicates that the intrinsic ligand is indeed behaving as a regulatory modulator capable of dynamically conferring two different modes of action.

Using fluorescence to track conformational change
To further examine the dynamics of the intrinsic ligand, Dai et al. (2018) introduce an unnatural fluorescent amino acid (Anap) at several positions in the vicinity of the intrinsic ligand and use an innovative combination of voltage-clamp fluorometry (VCF), patch-clamp fluorometry (PCF), and transition metal ion fluorescence resonance energy transfer (tmFRET) to simultaneously track both ionic current and conformational changes around the intrinsic ligand. Although VCF and PCF are useful for studying conformational dynamics of membrane proteins (Mannuzzu et al., 1996; Zheng and Zagotta, 2003), they do not necessarily provide spatial information about the direction and magnitude of movement, which is why the authors incorporate tmFRET into their experiments. Using these techniques, Dai et al. (2018) show that the intrinsic ligand undergoes conformational changes with kinetics identical to those of VDP. Additionally, they demonstrate that both VDP and the

rearrangement of the intrinsic-ligand environment are eliminated when the N-terminal Per-Arnt-Sim (PAS) cap of the eag domain is deleted. These findings show that the conformational change of the pore accompanying VDP is associated with structural rearrangements of the intrinsic ligand and that the PAS cap of the eag domain is required for these conformational changes to occur.

Context from recent KCNH structures
How exactly could the PAS cap of the eag domain be involved in conformational changes of the intrinsic ligand in the CNBHD? Could the interacting eag domain be engaging the ligand and directing its conformation in the binding pocket? The recent cryo–electron microscopy structures of two KCNH channels (rEAG1 and hERG1; Whicher and MacKinnon, 2016; Wang and MacKinnon, 2017) show how the intracellular domains are arranged relative to the voltage-sensing domain (VSD) and the pore. This lends support to the proposed mechanism by which the intrinsic ligand modulates channel function; the C-terminal CNBHDs are positioned under the C linkers, which are directly connected to the intracellular pore gate formed by the S6 helix. Together, the tetrameric assembly of C linkers and CNBHDs form a large intracellular gating ring structure directly underneath the pore (Fig. 2). The N-terminal eag domains, however, lie at the periphery of this intracellular gating ring, interacting with the outer face of the CNBHD where the intrinsic ligand sits. Additionally, the N-terminal PAS cap of each eag domain extends upward toward the VSD and is poised to interact with the VSD S4 and the C-linker gating ring (Whicher and MacKinnon, 2016; Wang and MacKinnon, 2017). As such, the regulatory eag domains are strategically located to communicate conformational changes from the VSD and the C linker down to the intrinsic ligand in the CNBHD. In turn, conformational changes in the CNBHD induced by structural rearrangements of the intrinsic ligand can be communicated directly up to the pore through the C linkers. In this context, the work by Dai et al. (2018) directly shows how rearrangements of the intracellular domains appear to mediate conformational changes of the intrinsic ligand in the CNBHD, thereby conferring an additional level of regulation to fine-tune the gating of the pore.

Exciting possibilities
The findings by Dai et al. (2018) provide fascinating mechanistic insight into how KCNH channels are regulated and, in particular, answer several questions about the regulatory role of the intrinsic ligand. The level of mechanistic insight into the modulatory action of the KCNH intrinsic ligand is unprecedented compared with intrinsic ligands proposed in other contexts (Sugi et al., 2007; Leppiniemi et al., 2012). Furthermore, the present work is intriguing from a phylogenetic perspective; the KCNH family of ion channels shares a common ancestor with both HCN and CNG channels, but it is unclear if cyclic nucleotide sensitivity was lost in KCNH channels and replaced with the intrinsic ligand or if the intrinsic ligand was replaced with cyclic nucleotide binding in the CNG/HCN channels. The regulatory eag domain, however, has been lost at least twice during evolution of the ERG family of channels (in *Drosophila*
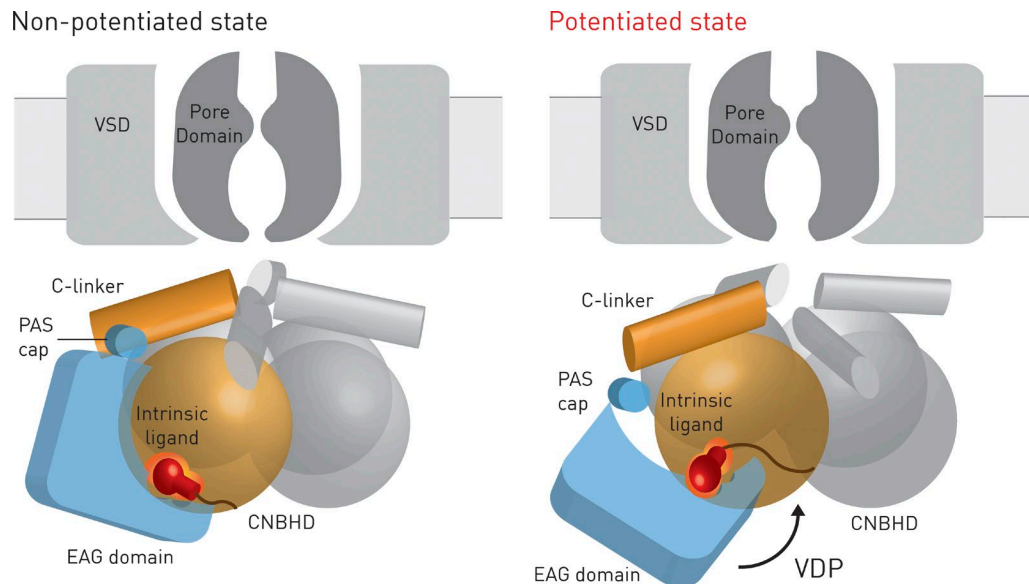
Figure 2. **Illustration of proposed conformational changes that occur in the intracellular domains and intrinsic ligand during VDP.** Conformational change of the intrinsic ligand that is associated with the rearrangement of the eag domain and CNBHD during VDP. The CNBHD and C-linker domains are depicted in tetrameric form with one subunit colored for easier visualization. For clarity, only one subunit of an interacting intersubunit eag domain with its N-terminal PAS cap is shown. Linkers between the intracellular domains and the VSD/pore are not depicted. The N-terminal eag domain is linked to the VSD, whereas the C-terminal CNBHD is linked to the pore domain via the C-linker domains.

melanogaster and *Caenorhabditis elegans* ERG; Martinson et al., 2014). Based on the current findings of Dai et al. (2018) that the eag domain is involved in a conformational switch of the intrinsic ligand, it would be interesting to see if the intrinsic ligand is present and plays a regulatory role in these channels lacking the eag domain.

Moreover, the intriguing finding that the channel can be regulated by an exogenous intrinsic-ligand peptide fragment could have important implications for the design of compounds that target this binding pocket for future treatment of heart and neurological diseases. Of direct relevance, a cardiac arrhythmia–associated mutation identified in the intrinsic ligand of hERG channels is known to impede function of the channel (Brelidze et al., 2013).

Overall, the study by Dai et al. (2018) elegantly demonstrates how a set of complementary experimental approaches can shed light on the complex regulatory mechanisms exerted by intracellular domains of ion channels. Applied to other ion channels, they will no doubt further advance our mechanistic understanding of how these proteins are regulated and fine-tuned while performing their vital physiological functions in the heart, brain, and beyond.

Acknowledgments

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