CACHD1: a new activity-modifying protein for voltage-gated calcium channels

Stephens, G. J. and Cottrell, G. S. (2019) CACHD1: a new activity-modifying protein for voltage-gated calcium channels. Channels, 13 (1). pp. 120-123. ISSN 1933-6969 doi: https://doi.org/10.1080/19336950.2019.1600968 Available at http://centaur.reading.ac.uk/83274/

It is advisable to refer to the publisher’s version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1080/19336950.2019.1600968

Publisher: Taylor & Francis

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading
Reading’s research outputs online
CACHD1: A new activity-modifying protein for voltage-gated calcium channels

Gary J. Stephens & Graeme S. Cottrell

To cite this article: Gary J. Stephens & Graeme S. Cottrell (2019) CACHD1: A new activity-modifying protein for voltage-gated calcium channels, Channels, 13:1, 120-123, DOI: 10.1080/19336950.2019.1600968

To link to this article: https://doi.org/10.1080/19336950.2019.1600968

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 13 Apr 2019.

Submit your article to this journal

View Crossmark data
Understanding the mechanisms that regulate the trafficking and expression of voltage-gated calcium channels (VGCCs) at presynaptic and potentially postsynaptic plasma membrane microdomains is of paramount importance. VGCCs are subdivided into two main families: high voltage-activated calcium channels (HVACCs) and low voltage-activated calcium channels (LVACCs) (reviewed [1]). HVACCs comprise Ca\(_{\alpha 1.1-4}\) (L-type current), Ca\(_{\alpha 2.1}\) (P/Q-type current), Ca\(_{\alpha 2.2}\) (N-type current) and Ca\(_{\alpha 2.3}\) (R-type current) VGCCs, whereas LVACCs comprise the Ca\(_{\alpha 3}\) (T-type current) family. The expression, assembly, localization and biophysical properties of HVACCs can be modulated by \(\alpha_2\delta\) and \(\beta\) auxiliary subunits. However, the modulation of Ca\(_{\alpha 3}\) channels by other proteins has remained less well defined. \(\alpha_2\delta\) and \(\beta\) subunits are not considered to be auxiliary subunits for Ca\(_{\alpha 3}\) subunits and consensus opinion is that Ca\(_{\alpha 3}\) can form fully functional channels alone (reviewed [2]). We have recently identified CACHD1 (Ca\(^{2+}\) channel and chemotaxis receptor (cache) domain containing protein 1), a protein with structural similarities to the \(\alpha_2\delta\) family, as a modulator of Ca\(_{\alpha 3}\) VGCC activity in expression systems and in native neurons [3]. Our study adds to other recently defined modulators of Ca\(_{\alpha 3}\) subunits which include endogenous molecules such as the actin binding protein kelch-like 1 (KLHL1), the Stac adaptor protein 1 (Stac1), the endoplasmic reticulum integral membrane protein, calnexin and the ubiquitin-specific protease, USP5 (reviewed [4]).

Primary sequence analysis reveals that although CACHD1 has limited sequence identity to \(\alpha_2\delta\) subunits, it similarly contains a VWA (von Willebrand Factor A) domain and putative bacterial chemosensory-like cache domains (Figure 1). However, there are a number of important differences compared to \(\alpha_2\delta\) subunits that will potentially affect CACHD1 function. For example, CACHD1 contains fewer predicted glycosylation sites, a variant metal ion-dependent adhesion site (MIDAS) motif (D\(^{234}\)xGxS) within the VWA domain and a variant gabapentin binding site (R\(^{213}\)-S-R). Of potential importance, CACHD1 is predicted to be a single transmembrane protein with a large intracellular C-terminal tail, unlike \(\alpha_2\delta\), which is reportedly a glycosphatidylinositol (GPI)-anchored protein (reviewed [2]). In our study, we demonstrated that CACHD1 promoted the cell surface localization of Ca\(_{\alpha 3.1}\) in a heterologous expression system [3]. Furthermore, using proximity ligation assays, we showed that CACHD1 and Ca\(_{\alpha 3.1}\) are <40 nm apart at the cell surface, supporting the formation of CACHD1:Ca\(_{\alpha 3.1}\) complexes at the plasma membrane. Our electrophoretic analyses indicated that CACHD1 migrated slower than its predicted molecular mass of 142 kDa, migrating at approximately ~170 kDa, possibly indicating post-translational glycosylation. Human CACHD1 was shown to cause clear effects on human Ca\(_{\alpha 3.1}\), Ca\(_{\alpha 3.2}\) and Ca\(_{\alpha 3.3}\) subunits, increasing peak current density with a corresponding increase in maximal conductance. In contrast, expression of \(\alpha_2\delta\) had no effect on T-type current under the same conditions. CACHD1 increased the open probability of the Ca\(_{\alpha 3.1}\) channel, pointing to mechanistic effects on the \(\alpha_1\) subunit. We further demonstrated functional effects of CACHD1 on native T-type current in rat hippocampal neurons. CACHD1 transfected neurons fired at a higher frequency than control neurons, an effect that was
prevented by the selective T-type current blocker TTA-P2. Furthermore, CACHD1 caused a significant increase in rebound firing frequency, a property mediated predominantly by T-type current; TTA-P2 similarly reversed this effect. Together, these data support a role for CACHD1 in increasing neuronal T-type current, which promotes an increase in action potential firing frequency and neuronal excitability. The future study of neurons from CACHD1 knockout mice will further advance knowledge of the physiological role of CACHD1.

A subsequent report has also identified a role for CACHD1 as a modulator of other VGCC subtypes, reporting effects on CaV2.2 [5]. Together, these studies suggest that CACHD1 may have broader therapeutic potential. However, although CACHD1 caused a significant increase in CaV2.2 current, this modulation was not as profound as seen for α2δ1; indeed, co-expression of CACHD1 caused a reduction in α2δ1-mediated actions on CaV2.2. These data indicate that CACHD1 may have a dominant negative-like effect on N-type calcium current and led to the suggestion that CACHD1 and α2δ1 may compete for the same structural motif on CaV2.2 [5], unlike the situation for CaV3 subunits. In our original studies, we did not detect any significant increase in Ca2+ current for human CaV2.2 co-expressed with human CACHD1 [6], in contrast to effects reported for rat CaV2.2 with zebrafish or rat CACHD1 [5]. Of further interest was that these CACHD1 orthologues had no effect on rat CaV2.1 [5]. Therefore, it may be that CACHD1 effects are dependent on the species of both CACHD1 and the α1 subunit.

The α2δ MIDAS motif (DxSxS) within its VWA domain plays a major role in their modulation of VGCC function (reviewed [2]). Approximately 46% of VWA domain-containing proteins contain a conserved MIDAS motif [7]. CACHD1 has a variant MIDAS motif (D234xGxS). Of functional importance, structural studies of VWA domains and biochemical analysis of a family of proteins called copines revealed that a perfectly conserved MIDAS motif is not required for metal ion binding [7]. Due to the difficulties of predicting non-contiguous structural motifs, such as cache domains, from
primary sequences, it remains undetermined if the downstream Thr and Asp residues in human CACHD1 contribute to a conserved MIDAS motif. However, one could speculate, based on our preliminary sequence alignments, that these residues are Thr$^{311}$ and Asp$^{338}$. Mutation of the Asp and Ser residues to Ala (AxAXa) in the α2δ1 MIDAS motif caused disruption of the interaction between α2δ1 and CaV2.2 and this interaction was also prevented by mutation of Asp$^{122}$ to Ala on CaV2.2 [5]; surprisingly, the D122A mutation did not prevent CACHD1-mediated enhancement of CaV2.2 currents. This data may indicate that CACHD1 does not interact with CaV2.2 in exactly the same way as α2δ1. Future biochemical and electrophysiological studies involving mutated forms of the CACHD1 variant MIDAS motif will shed light on its role in the regulation of the function of both CACHD1 and VGCCs. The electrophoretic mobility of rat CACHD1 was increased to its predicted level by treatment of whole cell lysates with the N-linked deglycosylating enzyme, PNGase F, indicating that rat CACHD1 is modified by N-linked glycosylation [5]. This study highlighted seven high potential N-linked glycosylation sites on rat CACHD1. Similar sequence analysis of human CACHD1 reveals one very high probability site (Asn$^{145}$), five other high probability Asn residues, with an additional three lower potential sites. Future experimentation will reveal precisely which residues are glycosylated and the role of glycosylation in the function of human CACHD1.

To date, CACHD1 and the α2δ isofoms are the only mammalian proteins known to contain bacterial chemosensory-like cache domains. Cache domains are the most important extracellular sensors in prokaryotes (reviewed [8]) and may represent potential drug targets. The current predicted CACHD1 structure, including two predicted cache domains, is based on extant Uniprot data (Q5VU97). For α2δ1, the initial description of a single cache domain (Uniprot data, P54289) has been revised to two and most recently to four domains following the latest crystal structure at 3.6 Å [9]. A similar crystal structure will be required to determine the number of cache domains in CACHD1, but the presence of multiple cache domains adds another dimension to the possible functionality of both α2δ and CACHD1.

With regard to our recent work, CACHD1 is predicted to act mechanistically to increase CaV3 channel open probability and promote T-type currents. Such physiological actions may have implications in targeting diseases involving aberrant neuronal firing. In particular, CaV3 channels have been proposed as therapeutic targets to combat pain and epilepsy (reviewed [4]). It is of particular interest that CACHD1 transcripts are highly expressed in mouse dorsal root ganglia [10], key transducers of nociceptive information. However, the therapeutic potential of CaV3-targeting drugs remains largely unrealized, as clinical drugs such as ethosuximide lack CaV3 selectivity and an earlier agent, mibebradil, was withdrawn due to serious drug interaction issues. It will be of interest to identify the functions of the domains and sequence motifs present in CACHD1 responsible for its effects on T-type currents and the sequences within CaV3 channels that may participate in the interaction with CACHD1. Targeting proteins such as CACHD1 that modulate CaV3 function may provide an attractive alternative to the direct blocking of the α1 subunit pore and we propose that CACHD1 represents an important new protein with excellent potential for future therapeutic targeting.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Gary J. Stephens http://orcid.org/0000-0002-8966-4238
Graeme S. Cottrell http://orcid.org/0000-0001-9098-7627

References

[1] Zamponi GW, Striessnig J, Koschak A, et al. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev. 2015;67:821–870.
[2] Dolphin AC. Calcium channel auxiliary alpha2delta and beta subunits: trafficking and one step beyond. Nat Rev Neurosci. 2012;13:542–555.
[3] Cottrell GS, Soubbrane CH, Hounshell JA, et al. CACHD1 is an alpha2delta-like protein that modulates CaV3 voltage-gated calcium channel activity. J Neurosci. 2018;38:9186–9201.
[4] Weiss N, Zamponi GW. T-type calcium channels: from molecule to therapeutic opportunities. Int J Biochem Cell Biol. 2019;108:34–39.
[5] Dahimene S, Page KM, Kadurin I, et al. The alpha2delta-like protein cachd1 increases N-type calcium currents and cell surface expression and competes with alpha2delta-1. Cell Rep. 2018;25:1610–1621 e1615.
[6] Soubrane CH, Stevens EB, Stephens GJ (2012) Expression and functional studies of the novel CNS protein CACHD1. Proceedings of The Physiological Society; Edinburgh; Vol. 27, PC74
[7] Whittaker CA, Hynes RO. Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. Mol Biol Cell. 2002;13:3369–3387.
[8] Anantharaman V, Aravind L. Cache - a signaling domain common to animal Ca2+-channel subunits and a class of prokaryotic chemotaxis receptors. Trends Biochem Sci. 2000;25:535–537.
[9] Wu J, Yan Z, Li Z, et al. Structure of the voltage-gated calcium channel CaV1.1 at 3.6 Å resolution. Nature. 2016;537:191–196.
[10] Ray P, Torck A, Quigley L, et al. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. Pain. 2018;159:1325–1345.