REVIEW ARTICLE

Advances in understanding of structure, function and plasticity of HCN channels

Sukant Khurana*

Indian Institute of Science Education and Research Kolkata, Mohanpur, Nadia 741 246, India and
CSIR-Central Drug Research Institute, Lucknow 226 031, India

Hyperpolarization-activated, cyclic nucleotide-sensitive, cation non-selective (HCN) channels are gaining attention in recent years. These ion-channels are gated by two factors: voltage, specifically hyperpolarization and some subunits, more than others, by cyclic nucleotides. They conduct both sodium and potassium ions, and regulate many functions in both neuronal and non-neuronal cells. In neurons, HCN channel functions range from setting resting potential, synaptic normalization, gain control, after-hyperpolarization, setting responses in dendrites, mediating cannabinoid role in neuronal plasticity to the gating of plasticity. Emerging properties of circuits expressing HCN channels manifest in the form of various kinds of functions like brain rhythms, perception, spatial learning, executive memory, epilepsy, ataxia, depression, sound localization, anxiety, etc. We are beginning to understand the roles of these channels in non-neuronal cells and we would discover more such roles in the future. Following ischaemia, there is HCN overexpression in the reactive astrocytes. There is an altered expression of HCN channels in few tissues in diabetic animal models. Recent evidence suggests that HCN channels are also involved in uterine contractions and renal functioning. This review focuses on the function, structure, interacting proteins and developmental plasticity that enable the versatility of HCN channels.

Keywords: Developmental plasticity, HCN channels, interacting proteins.

Hyperpolarization-activated, cyclic nucleotide-sensitive, cation non-selective (HCN) channels now occupy the centre-stage of ion-channel research[1]. In this review, I will briefly describe the structure and function of HCN channels and then elaborate on the developments in the regulation and developmental plasticity of these channels.

Physiology and functions of HCN

HCN channels allow current that opens on hyperpolarization (I_h). HCN channels are crucial in cardiac pacemaking, learning and memory, neuronal gain, establishing resting potential, and many other physiological functions. The molecular substrates for I_h are HCN1 to HCN4 in mammals[2–4]. HCN channels, unlike most cationic channels, have a higher probability of opening in response to hyperpolarization[5–9]. They have a reversal potential in several neurons of around –30 to –35 mV, and this varies across cell types. However, it is not clear whether the differences in reversal potential across different cells are due to any differences in conductance or those in the intracellular potassium concentration in different cells[10,11]. Given a depolarized reversal value than the resting potential, the opening of HCN channels results in an inward current for most voltage ranges. Hormones and transmitters can alter the voltage dependence of these channels such as modulation by norepinephrine of I_h (refs 10, 12, 13). I_h was discovered in the sinoatrial node of the heart and in the Purkinje neurons[5,14]. In the cardiac tissue, this current was originally denoted as I_f (f, funny)[7]. Now we know that for both I_h and I_f, the same channel types are involved[1,15].

These channels play diverse roles in different brain regions and neuron types. In the thalamo-cortical neurons, HCN channels play a pacemaking role[16,17]. In cerebellar Purkinje neurons I_h opposes long-lasting inhibition, thus enabling burst firing[18]. HCN channel activity ensures that membrane potential in many neurons is in the range that allows for other channels to control pacemaking[19]. As more roles of cerebellum are being discovered, I suspect that more research would be focused on understanding the complex role of HCN in various cerebellum cell types, as evinced by the recent work on granule cells[20]. Gradient of these channels in dendrites is also known to be involved in synaptic normalization by hyperpolarization due to turning-off of a depolarizing influence and also by providing increasing shunt with increasing distance from soma[21]. HCN channels might emerge as important interactors, which connect ion-channel plasticity with cytoskeleton activity[22], acting as a signalling intermediate between cytoskeleton synaptic plasticity[23,24]. Distance-dependent sub-threshold resonance frequency of neurons increases with increase in I_h which can play a selective role in amplifying inputs of select range of frequencies[25,26]. This line of work and its integration with the rhythms of brain and brain maps would be an exciting area of work on HCN channels. HCN channels may play a major role in governing internal time delays within neurons as an additional mechanism for

*e-mail: sukantkhurana@gmail.com

synaptic integration[25]. In auditory processing where time delays play a central role in computation, this interaction of I_h with low-voltage activated potassium current results in a constant output of brainstem auditory neurons[11].

HCN subunits have been studied using *in situ* hybridization[27–29] and immuno-histochemistry approaches. Instead of subunit differences across brain regions, physiological differences in I_h across the brain are due to associated proteins and signalling. That said, subunit specificity is important because of the underlying differences in the expression pattern of various subunits and likely association with different modulator proteins. HCN1 promotes the ability to learn new motor tasks, but it inhibits spatial learning and memory[30]. HCN2 plays an important role in the regulation of cellular excitability, motion control and synus node functioning because its knockout results in absence epilepsy, ataxia and sinus node dysfunction[31]. HCN4 is vital for cardiac development; its knockout fails to have sinoatrial node-like action potential in mice and such knockouts are embryonic lethal[31–33]. HCN1 also regulates the size and stability of hippocampal place fields[34]. HCN1 knockdown results in enhancement of dorsal hippocampal activity, which manifests in states akin to anxiolytic- and antidepressant-like behaviours[35], raising hopes of targeting HCN1 for treatment of depression in the future. Chronic unpredictable stress results in an increase in I_h near soma in dorsal CA1 neurons, as measured by molecular and patch clamp approaches[36]. This is not the case for acute stress. Importance of HCN channels in depression studies is obvious from the elimination of an important protein involved in its localization, tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b). Knockout of TRIP8b in pyramidal neuron dendrites in mice, manifests, in addition to motor learning deficits, in enhanced resistance to multiple tasks of behavioural despair, a known measure of antidepressant efficacy[37].

The single-channel conductance of HCN is very low (<1 pS), rendering single-channel recordings problematic with the current state-of-the-art in eletrophysiology[9,38], and claims of such recordings have been controversial[39]. In our study for the same channel composition, kinetics changed with changes in intracellular signalling, indicating that like voltage of activation, kinetics can be easily modulated[40]. The activation time courses of HCN channels vary across cell types[41].

Regulation of HCN channels

HCN channels can be extensively modulated, making them play several roles in the brain. Membrane phosphoinositides, including phosophatidylinositol-4, 5-bisphosphate (PIP_2)[16,40,42,44] and extracellular and intracellular chloride[45], non-receptor tyrosine kinase c-Srcl[46,47] and p38-MAP kinase (P38MAPK)[40,48] are few of the several modulators of HCN channels.

The MinK-related protein, MiRP1 is an established auxiliary subunit of the HERG delayed rectifier K^+ channel[49–51], which increases the current and activation of HCN2 (refs 52, 53), but it has no influence on the voltage-dependence of HCN2 activation. For HCN4, MiRP1 has a few similar as well as different effects compared to its influence on HCN2; it increases the current, slows down kinetics and hyperpolarizes the activation curve of this channel[54].

HCN2 has been reported to interact with scaffold proteins Mint2 and S-SCAM[55]. Among all HCN subunits, only HCN2 selectively interacts with tamalin[55]. Filamin A can selectively interact with one subunit of HCN, i.e. HCN1 (ref. 22). Filamin causes clustering of channels and slows down the kinetics of HCN1 (ref. 22). In expression systems, HCN channels are also shown to interact with lipid rafts, caveoli and their responses have been tested as a result of cholesterol depletion[8,56].

Trip8b has been studied for several HCN regulation activities[57]. Different splice variants of Trip8b can have opposite effects on HCN channel activity and are responsible for hastening of HCN2 and cyclic AMP insensitivity of HCN2 (refs 57–61). Trip8b interacts through C-terminal domain regions of HCN channels[61,62], regulating channel trafficking[61,63,64] along with gating. Axonal trafficking of HCN1 in perforant path involves specific expression of Trip8b isoforms[65]. Hetero-multimerization can increase HCN physiological variety and is well known in invertebrate channels[66]. Biochemical regulation of HCN channels can also play a major role, for example, signalling related to HCN channels strongly influences the sub-millisecond time processing ability of sound localization neurons[40].

Developmental changes in I_h

With development several changes take place in the HCN channels. In hippocampus there is a reported increase in cyclic AMP with development but decrease in subunits proportion, which are sensitive to it[68]. This was further explored at RNA and protein levels to narrow down to HCN1 increase, HCN4 reduction and HCN2 stability[29]. In rat hippocampus, conductance of HCN channels triples with age in CA3 neurons and quintuples in the CA1 neurons, but if this is normalized for membrane area, it corresponds to maximum HCN channel density at PND5 (ref. 69). Different studies show different subunit changes. The differences across studies are further complicated by only marginally satisfactory antibody specificity. In the intra-cardiac neurons there is independent modulation of total conductance and biochemical modulation and also an interesting trend of decrease in conductance with age[69], unlike most other neurons, which show an increase. In auditory brainstem sound localization neurons of medial superior olive, a 13-fold increase in maximal HCN conductance along with a >10-fold acceleration of kinetics,

and a 30 mV depolarizing shift in the voltage dependence of activation were observed. In this case, it is likely that the major change was intracellular signalling of PIP2, cAMP and P38 MAPkinase and no subunit transition[40]. This shows that there are many ways to modulate HCN channels. This study demonstrates that biological systems can accomplish a change in HCN channel function not just by subunit change, but simply by biochemical modulation. It is not a surprise, given the plasticity of HCN channels, that they are involved in many different roles in different neurons. It is the plasticity of HCN channels that makes them particularly attractive targets of research and drug discovery. It has been a quite journey for HCN channel research in the last few years, but I believe that the best is yet to come. In future studies, I hope that there will be a better synergy of computational, electrophysiological and molecular approaches, and of putting cellular functions in the context of network activity and how it shapes behaviour. With single-cell RNA sequence approaches[70] and metabolomics[71] information now becoming commonplace, better analysis of subunit composition and biochemical modulation and employment of novel methods to complement traditional pharmacology and immunohistochemistry for better understanding of mechanisms of channel modulation are possible.

Recent studies and future directions

It is no surprise that in the last few years, the role of HCN channels in several more diseases has been discovered. Also interestingly, novel modulators of HCN channels and roles in cellular functioning are being identified, making it an exciting research area.

HCN channels are involved in neuropathic pain[72]. HCN2 channels are fast emerging as important in pain signalling and several new modulators of their signalling are being identified, which should one day provide a target for pain-related drug discovery[73]. Increased HCN channel activity in the ventral posterolateral nucleus is responsible for neuropathic and inflammatory pain conditions[74]. The cellular mechanism of neuropeptide S (NPS), an endogenous anxiolytic is not clear, but recent experiments show that it inhibits HCN1 in the amygdala of rat through activation of NPS receptor (NPSR); this effect is through ERK1/2 phosphorylation in a subset of pyramidal-like neurons located in the medial amygdala[75]. In addition, HCN1 and HCN2 are involved in neuropathic pain in peripheral neurons[76] in rodent models, suggesting HCN as a promising channel type for chronic pain research. Given that chronic pain has evaded serious medication, identification of the role of HCN channels provides a promising avenue for drug-discovery.

The role of HCN channels in synaptic transmission is just coming to the fore. Presynaptic HCN channels can both inhibit and enhance synaptic transmission based on a milieu of other ion channels present in the terminal. In calyx of Held, giant auditory brainstem synapse, HCN channels have been found to regulate presynaptic vesicular trafficking by regulating the amount of sodium ion[77]. HCN1 is expressed in some presynaptic terminal within the mature entorhinal (EC) cortex controls both spontaneous as well as synchronous evoked release[78]. In larvae of *Drosophila melanogaster* motor terminals, HCN channels regulate neurotransmitter release by serotonin[79]. Genetic disruption of the sole HCN gene reduces the amplitude of the evoked response at the neuromuscular junction (NMJ) of third instar larvae by decreasing the number of released vesicles and partial loss-of-function mutant adult flies have impaired locomotion, suggesting that the HCN channel may contribute to coordinated movement[79].

While the role of HCN channels in learning and memory has been well established[30], until recently, it was not clear that it can play a role in some kind of autism as well[80]. Recently, the role of HCN channels in depression is also coming to the fore[36,81].

Reduction of HCN channels in thalamus and cortex (through TRIP8b knockout) results in a model of absence epilepsy[82] and recent work has shown these channels to be a good drugable targets for epilepsy treatment[31,83,84].

Auditory roles of these channels are also becoming clearer with new information, with the latest study showing their crucial role in tinnitus too[85]. Interestingly, more modulators of HCN continue to be found[86–88].

Very few studies are available on the role of HCN channels in addiction[89,90] but given the role of these channels and addictive agents in the regulation of neuronal excitability, HCN channels as targets of addiction research seem to be under-explored. HCN channels in nucleus accumbens modulate methamphetamine self-administration in rats[89]. Cocaine sensitization increases HCN2 expression in structures of the mesocorticolimbic system[90].

HCN channels have been known to be involved in diseases of non-neuronal cells, noticeably cardiac cells, but now even other non-neuronal cells are being found in HCN pathogenesis. HCN channels could provide interesting medicine target in diabetes too. Streptozotocin (STZ)-induced diabetic rats have a lower intrinsic heart rate, an inferior leading pacemaker site, reduced sinoatrial node (SAN) conduction velocity and diastolic depolarization slope, and a longer action potential duration than in the control. The transcripts and proteins of HCN2 and HCN4 in diabetic SAN are reduced, suggesting possible involvement of HCN channels in diabetes-related cardiac problems[91]. In diabetes, bladder Cajal-like interstitial cells are impaired and recently, HCN channels expression associated with caveolae and caveolin was found to be decreased too, suggesting this as one of the molecular mechanisms underlying diabetic bladder problems[92]. Pregnant uterine contractility has also been reported to be modulated by HCN channels[93] and I hope that these channels offer a drugable target for preterm labour.

To summarize, HCN channels are proving to be involved in a wide variety of functions and a rich target for drug discovery. A richer crosstalk between different research areas and integration of computation and experimentation are needed to promote a fuller understanding of these fascinating channels.

1. Santoro, B. and Tibbs, G. R., The HCN gene family: molecular basis of the hyperpolarization-activated pacemaker channels. *Ann. NY Acad. Sci*., 1999, **868**, 741–764.
2. Santoro, B., Grant, S. G., Bartsch, D. and Kandel, E. R., Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homology to eag and cyclic nucleotide-gated channels. *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 14815–14820.
3. Ludwig, A., Zong, X., Jeglitsch, M., Hofmann, F. and Biel, M., A family of hyperpolarization-activated mammalian cation channels. *Nature*, 1998, **393**, 587–591.
4. Santoro, B. *et al.*, Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell*, 1998, **93**, 717–729.
5. DiFrancesco, D., A new interpretation of the pace-maker current in calf Purkinje fibres. *J. Physiol*., 1981, **314**, 359–376.
6. DiFrancesco, D., Characterization of the pace-maker current kinetics in calf Purkinje fibres. *J. Physiol.*, 1984, **348**, 341–367.
7. Barbuti, A. and DiFrancesco, D., Control of cardiac rate by 'funny' channels in health and disease. *Ann. NY Acad. Sci.*, 2008, **1123**, 213–223.
8. Barbuti, A., Baruscotti, M. and DiFrancesco, D., The pacemaker current: from basics to the clinics. *J. Cardiovasc. Electrophysiol.*, 2007, **18**, 342–347.
9. DiFrancesco, D., Characterization of single pacemaker channels in cardiac sinoatrial node cells. *Nature*, 1986, **324**, 470–473.
10. Maccaferri, G. and McBain, C. J., The hyperpolarization-activated current (I_h) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. *J. Physiol.*, 1996, **497**(1), 119–130.
11. Khurana, S., Remme, M. W. H., Rinzel, J. and Golding, N. L., Dynamic interaction of Ih and IK-LVA during trains of synaptic potentials in principal neurons of the medial superior olive. *J. Neurosci*., 2011, **31**, 8936–8947.
12. Cao, D.-N., Song, R., Zhang, S.-Z., Wu, N. and Li, J., Nucleus accumbens hyperpolarization-activated cyclic nucleotide-gated channels modulate methamphetamine self-administration in rats. *Psychopharmacology* (*Berl*), 2016, **233**, 3017–3029.
13. DiFrancesco, D. and Mangoni, M., Modulation of single hyperpolarization-activated channels (i(f)) by cAMP in the rabbit sinoatrial node. *J. Physiol*., 1994, **474**, 473–482.
14. Hagiwara, N. and Irisawa, H., Modulation by intracellular Ca^{2+} of the hyperpolarization-activated inward current in rabbit single sino-atrial node cells. *J. Physiol*., 1989, **409**, 121–141.
15. Pape, H. C., Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu. Rev. Physiol*., 1996, **58**, 299–327.
16. Munsch, T. and Pape, H. C., Modulation of the hyperpolarization-activated cation current of rat thalamic relay neurones by intracellular pH. *J. Physiol*., 1999, **519**(Pt 2), 493–504.
17. Leist, M. *et al*., Two types of interneurons in the mouse lateral geniculate nucleus are characterized by different *h*-current density. *Sci. Rep.*, 2016, **6**, 24904.
18. Williams, S. R., Christensen, S. R., Stuart, G. J. and Häusser, M., Membrane potential bistability is controlled by the hyperpolarization-activated current I(H) in rat cerebellar Purkinje neurons *in vitro*. *J. Physiol.*, 2002, **539**, 469–483.
19. Robinson, R. B. and Siegelbaum, S. A., Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu. Rev. Physiol.*, 2003, **65**, 453–480.
20. Zúñiga, R., González, D., Valenzuela, C., Brown, N. and Zúñiga, L., Expression and cellular localization of HCN channels in rat cerebellar granule neurons. *Biochem. Biophys. Res. Commun*., 2016, **478**, 1429–1435.
21. Magee, J. C., Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J. Neurosci*., 1998, **18**, 7613–7624.
22. Gravante, B. *et al.*, Interaction of the pacemaker channel HCN1 with filamin A. *J. Biol. Chem*., 2004, **279**, 43847–43853.
23. Beaumont, V. and Zucker, R. S., Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic Ih channels. *Nature Neurosci.*, 2000, **3**, 133–141.
24. Beaumont, V., Zhong, N., Froemke, R. C., Ball, R. W. and Zucker, R. S., Temporal synaptic tagging by I_h activation and actin: involvement in long-term facilitation and cAMP-induced synaptic enhancement. *Neuron*, 2002, **33**, 601–613.
25. Narayanan, R. and Johnston, D., The *h* channel mediates location dependence and plasticity of intrinsic phase response in rat hippocampal neurons. *J. Neurosci*., 2008, **28**, 5846–5860.
26. Narayanan, R. and Johnston, D., Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. *Neuron*, 2007, **56**, 1061–1075.
27. Moosmang, S., Biel, M., Hofmann, F. and Ludwig, A., Differential distribution of four hyperpolarization-activated cation channels in mouse brain. *Biol. Chem*., 1999, **380**, 975–980.
28. Santoro, B. *et al.*, Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J. Neurosci*., 2000, **20**, 5264–5275.
29. Brewster, A. L. *et al.*, Quantitative analysis and subcellular distribution of mRNA and protein expression of the hyperpolarization-activated cyclic nucleotide-gated channels throughout development in rat hippocampus. *Cereb. Cortex*, 2007, **17**, 702–712.
30. Nolan, M. F. *et al.*, A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell*, 2004, **119**, 719–732.
31. Herrmann, S., Stieber, J. and Ludwig, A., Pathophysiology of HCN channels. *Pflügers Arch. – Eur. J. Physiol*., 2007, **454**, 517–522.
32. Stieber, J. *et al*., The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 15235–15240.
33. Zong, X., Stieber, J., Ludwig, A., Hofmann, F. and Biel, M., A single histidine residue determines the pH sensitivity of the pacemaker channel HCN2. *J. Biol. Chem*., 2001, **276**, 6313–6319.
34. Hussaini, S. A., Kempadoo, K. A., Thuault, S. J., Siegelbaum, S. A. and Kandel, E. R., Increased size and stability of CA1 and CA3 place fields in HCN1 knockout mice. *Neuron*, 2011, **72**, 643–653.
35. Kim, C. S., Chang, P. Y. and Johnston, D., Enhancement of dorsal hippocampal activity by knockdown of HCN1 channels leads to anxiolytic- and antidepressant-like behaviors. *Neuron*, 2012, **75**, 503–516.
36. Kim, C. S., Brager, D. H. and Johnston, D., Perisomatic changes in h-channels regulate depressive behaviors following chronic unpredictable stress. *Mol. Psychiatry*, 2017; doi:10.1038/mp.2017.28.
37. Lewis, A. S. *et al.*, Deletion of the hyperpolarization-activated cyclic nucleotide-gated channel auxiliary subunit TRIP8b impairs hippocampal Ih localization and function and promotes antidepressant behavior in mice. *J. Neurosci.*, 2011, **31**, 7424–7440.
38. Kole, M. H. P., Hallermann, S. and Stuart, G. J., Single Ih channels in pyramidal neuron dendrites: properties, distribution, and

impact on action potential output. *J. Neurosci*., 2006, **26**, 1677–1687.
39. Simeone, T. A., Rho, J. M. and Baram, T. Z., Single channel properties of hyperpolarization-activated cation currents in acutely dissociated rat hippocampal neurones. *J. Physiol*., 2005, **568,** 371–380.
40. Khurana, S. *et al.*, An essential role for modulation of hyperpolarization-activated current in the development of binaural temporal precision. *J. Neurosci*., 2012, **32**, 2814–2823.
41. Wahl-Schott, C. and Biel, M., HCN channels: structure, cellular regulation and physiological function. *Cell. Mol. Life Sci.*, 2009, **66**, 470–494.
42. Pian, P., Bucchi, A., Robinson, R. B. and Siegelbaum, S. A., Regulation of gating and rundown of HCN hyperpolarization-activated channels by exogenous and endogenous PIP2. *J. Gen. Physiol*., 2006, **128**, 593–604.
43. Zolles, G. *et al.*, Pacemaking by HCN channels requires interaction with phosphoinositides. *Neuron*, 2006, **52**, 1027–1036.
44. Stevens, D. R. *et al.*, Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. *Nature*, 2001, **413**, 631–635.
45. Wahl-Schott, C., Baumann, L., Zong, X. and Biel, M., An arginine residue in the pore region is a key determinant of chloride dependence in cardiac pacemaker channels. *J. Biol. Chem.*, 2005, **280**, 13694–13700.
46. Zong, X. *et al.*, A novel mechanism of modulation of hyperpolarization-activated cyclic nucleotide-gated channels by Src Kinase. *J. Biol. Chem*., 2005, **280**, 34224–34232.
47. Li, C.-H. *et al.*, Src tyrosine kinase alters gating of hyperpolarization-activated HCN4 pacemaker channel through Tyr531. *Am. J. Physiol. – Cell Physiol*., 2008, **294**, C355–C362.
48. Poolos, N. P., Bullis, J. B. and Roth, M. K., Modulation of h-channels in hippocampal pyramidal neurons by p38 mitogen-activated protein kinase. *J. Neurosci.*, 2006, **26**, 7995–8003.
49. Abbott, G. W. *et al.*, MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell*, 1999, **97**, 175–187.
50. Tinel, N. *et al.* M-type KCNQ2-KCNQ3 potassium channels are modulated by the KCNE2 subunit. *FEBS Lett*., 2000, **480**, 137–141.
51. Zhang, M., Jiang, M. and Tseng, G. N., MinK-related peptide 1 associates with Kv4.2 and modulates its gating function: potential role as beta subunit of cardiac transient outward channel? *Circ. Res*., 2001, **88**, 1012–1019.
52. Yu, H. *et al.* MinK-related peptide 1: a beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. *Circ. Res*., 2001, **88**, E84–E847.
53. Qu, J. *et al.*, MiRP1 modulates HCN2 channel expression and gating in cardiac myocytes. *J. Biol. Chem*., 2004, **279**, 43497–43502.
54. Decher, N., Bundis, F., Vajna, R. and Steinmeyer, K., KCNE2 modulates current amplitudes and activation kinetics of HCN4: influence of KCNE family members on HCN4 currents. *Pflugers Arch. – Eur. J. Physiol.*, 2003, **446**, 633–640.
55. Kimura, K., Kitano, J., Nakajima, Y. and Nakanishi, S., Hyperpolarization-activated, cyclic nucleotide-gated HCN2 cation channel forms a protein assembly with multiple neuronal scaffold proteins in distinct modes of protein-protein interaction. *Genes Cells*, 2004, **9**, 631–640.
56. Barbuti, A. *et al.*, Localization of pacemaker channels in lipid rafts regulates channel kinetics. *Circ. Res*., 2004, **94**, 1325–1331.
57. Santoro, B., Wainger, B. J. and Siegelbaum, S. A., Regulation of HCN channel surface expression by a novel C-terminal protein–protein interaction. *J. Neurosci*., 2004, **24**, 10750–10762.
58. Lewis, A. S. *et al.*, Alternatively spliced isoforms of TRIP8b differentially control h channel trafficking and function. *J. Neurosci.*, 2009, **29**, 6250–6265.
59. Santoro, B. *et al.*, TRIP8b splice variants form a family of auxiliary subunits that regulate gating and trafficking of HCN channels in the brain. *Neuron*, 2009, **62**, 802–813.
60. Zolles, G. *et al.*, Association with the auxiliary subunit PEX5R/Trip8b controls responsiveness of HCN channels to cAMP and adrenergic stimulation. *Neuron*, 2009, **62**, 814–825.
61. Santoro, B. *et al.*, TRIP8b regulates HCN1 channel trafficking and gating through two distinct C-terminal interaction sites. *J. Neurosci.*, 2011, **31**, 4074–4086.
62. Bankston, J. R. *et al*., Structure and stoichiometry of an accessory subunit TRIP8b interaction with hyperpolarization-activated cyclic nucleotide-gated channels. *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 7899–7904.
63. Han, Y. *et al.*, Trafficking and gating of hyperpolarization-activated cyclic nucleotide-gated channels are regulated by interaction with tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b) and cyclic AMP at distinct sites. *J. Biol. Chem*., 2011, **286**, 20823–20834.
64. Huang, Z. *et al.*, TRIP8b-independent trafficking and plasticity of adult cortical presynaptic HCN1 channels. *J. Neurosci*., 2012, **32**, 14835–14848.
65. Wilkars, W. *et al.*, Regulation of axonal HCN1 trafficking in perforant path involves expression of specific TRIP8b isoforms. *PLOS ONE*, 2012, **7**, e32181.
66. Gisselmann, G. *et al.*, Molecular and functional characterization of an I (h)-channel from lobster olfactory receptor neurons. *Eur. J. Neurosci*., 2005, **21**, 1635–1647.
67. Surges, R. *et al.*, Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. *Eur. J. Neurosci*., 2006, **24**, 94–104.
68. Vasilyev, D. V. and Barish, M. E., Postnatal development of the hyperpolarization-activated excitatory current Ih in mouse hippocampal pyramidal neurons. *J. Neurosci*., 2002, **22**, 8992–9004.
69. Hogg, R. C., Harper, A. A. and Adams, D. J., Developmental changes in hyperpolarization-activated currents I(h) and I(K(IR)) in isolated rat intracardiac neurons. *J. Neurophysiol*., 2001, **86**, 312–320.
70. Westermann, A. J., Gorski, S. A. and Vogel, J., Dual RNA-seq of pathogen and host. *Nature Rev. Microbiol*., 2012, **10**, 618–630.
71. Ram, P. T., Mendelsohn, J. and Mills, G. B., Bioinformatics and systems biology. *Mol. Oncol*., 2012, **6**, 147–154.
72. Tibbs, G. R., Posson, D. J. and Goldstein, P. A., Voltage-gated ion channels in the PNS: novel therapies for neuropathic pain? *Trends Pharmacol. Sci*., 2016, **37**, 522–542.
73. Emery, E. C., Young, G. T. and McNaughton, P. A., HCN2 ion channels: an emerging role as the pacemakers of pain. *Trends Pharmacol. Sci*., 2012, **33**, 456–463.
74. Ding, W. *et al.*, Inhibition of HCN channel activity in the thalamus attenuates chronic pain in rats. *Neurosci. Lett.*, 2016, **631**, 97–103.
75. Zhang, S. *et al.*, Neuropeptide S modulates the amygdaloidal HCN activities (Ih) in rats: implication in chronic pain. *Neuropharmacology*, 2016, **105**, 420–433.
76. Liu, H., Zhou, J., Gu, L. and Zuo, Y., The change of HCN1/HCN2 mRNA expression in peripheral nerve after chronic constriction injury induced neuropathy followed by pulsed electromagnetic field therapy. *Oncotarget*, 2016, **8**, 1110–1116.
77. Huang, H. and Trussell, L. O., Presynaptic HCN channels regulate vesicular glutamate transport. *Neuron*, 2014, **84**, 340–346.
78. Huang, Z., Li, G., Aguado, C., Lujan, R. and Shah, M. M., HCN1 channels reduce the rate of exocytosis from a subset of cortical synaptic terminals. *Sci. Rep*., 2017, **7**, 40257.
79. Hegle, A. P. *et al.*, The Ih channel gene promotes synaptic transmission and coordinated movement in *Drosophila melanogaster*. *Front. Mol. Neurosci*., 2017, **10**, 41.

80. Yi, F. *et al.*, Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in human neurons. *Science*, 2016, **352**, aaf2669.
81. Han, Y. *et al.*, HCN-channel dendritic targeting requires bipartite interaction with TRIP8b and regulates antidepressant-like behavioral effects. *Mol. Psychiatry*, 2017, **22**, 458–465.
82. Heuermann, R. J. *et al.*, Reduction of thalamic and cortical Ih by deletion of TRIP8b produces a mouse model of human absence epilepsy. *Neurobiol. Dis.*, 2016, **85**, 81–92.
83. Shah, M. M., Huang, Z. and Martinello, K., HCN and KV7 (M-) channels as targets for epilepsy treatment. *Neuropharmacology*, 2013, **69**, 75–81.
84. Shah, M. M., Hyperpolarization-activated cyclic nucleotide-gated channel currents in neurons. *Cold Spring Harbor. Protoc*., 2016; pdb.top087346.
85. Li, S., Kalappa, B. I. and Tzounopoulos, T., Noise-induced plasticity of KCNQ2/3 and HCN channels underlies vulnerability and resilience to tinnitus. *Elife*, 2015, **4**.
86. Kopp-Scheinpflug, C., Pigott, B. M. and Forsythe, I. D., Nitric oxide selectively suppresses IH currents mediated by HCN1-containing channels. *J. Physiol*., 2015, **593**, 1685–1700.
87. Ko, K. W., Rasband, M. N., Meseguer, V., Kramer, R. H. and Golding, N. L., Serotonin modulates spike probability in the axon initial segment through HCN channels. *Nature Neurosci*., 2016, **19**, 826–834.
88. Carlson, A. E., Rosenbaum, J. C., Brelidze, T. I., Klevit, R. E. and Zagotta, W. N., Flavonoid regulation of HCN2 channels. *J. Biol. Chem*., 2013, **288**, 33136–33145.
89. Cao, D.-N., Song, R., Zhang, S.-Z., Wu, N. and Li, J., Nucleus accumbens hyperpolarization-activated cyclic nucleotide-gated channels modulate methamphetamine self-administration in rats. *Psychopharmacology*, 2016, **233**, 3017–3029.
90. Santos-Vera, B. *et al.*, Cocaine sensitization increases I h current channel subunit 2 (HCN_2) protein expression in structures of the mesocorticolimbic system. *J. Mol. Neurosci.*, 2013, **50**, 234–245.
91. Huang, X. *et al.*, Reduced expression of HCN channels in the sinoatrial node of streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol*., 2017, **95**, 586–594.
92. Dong, X. *et al.*, Interaction of caveolin-3 and HCN is involved in the pathogenesis of diabetic cystopathy. *Sci. Rep*., 2016, **6**, 24844.
93. Alotaibi, M., Kahlat, K., Nedjadi, T. and Djouhri, L., Effects of ZD7288, a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker, on term-pregnant rat uterine contractility *in vitro*. *Theriogenology*, 2017, **90**, 141–146.

Received 16 September 2016; revised accepted 14 August 2017

doi: 10.18520/cs/v114/i03/462-467