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REVIEW ARTICLE

Characteristics of HCN Channels and Their Participation in Neuropathic Pain

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Abstract Neuropathic pain is induced by the injury to nervous systems and characterized by hyperalgesia, allodynia and spontaneous pain. The underlying mechanisms include peripheral and central sensitization resulted from neuronal hyperexcitability. A number of ion channels are considered to contribute to the neuronal hyperexcitability. Here, we particularly concentrate on an interesting ion channel, hyperpolarization-activated cyclic nucleotide gated (HCN) channels. We overview its biophysical properties, physiological functions, followed by focusing on the current progress in the study of its role in the development of neuropathic pain. We attempt to provide a comprehensive review of the potential valuable target, HCN channels, in the treatment of neuropathic pain.

Keywords Hyperpolarization-activated cyclic nucleotide gated cation channels (HCN) · I_h · Neuropathic pain · Ectopic discharges · Peripheral sensitization · ZD7288

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Introduction

Chronic neuropathic pain, induced by injury to the nervous system, is characterized by spontaneous pain, hyperalgesia and allodynia. The mechanisms underlying the pathogenesis of neuropathic pain are complicated. Both peripheral and central sensitizations are involved. Spontaneous ectopic discharges of injured primary sensory neurons and sensitization of uninjured afferents are believed to be the major peripheral components. Centrally, enhanced nociceptive synaptic transmissions in the spinal dorsal horn and changed descending regulations in supraspinal central nervous systems are critical for the development of neuropathic pain [1–3].

Generally, the nociceptive transmission pathways exhibit hyperexcitability after injury of the nervous system, which exaggerates normal or noxious stimuli and induces chronic, abnormal painful reaction [4]. Ion channels are the primary determinants of neuronal excitability, a wide range of ion channels including sodium channels, potassium channels, calcium channels [5–7] are of great interest. Recently, hyperpolarization-activated cyclic nucleotide gated cation (HCN) channels have been identified as an important contributor and a valuable target for the treatment of neuropathic pain [8]. A series of studies from several research groups, including ours, have been

examining their roles in neuropathic pain on a variety of animal models. In this review, we will give a brief introduction of this channel, followed by an extensive discussion about the current progress in the study of its role in the development of neuropathic pain.

Overview of HCN Channels

Hyperpolarization-activated cyclic nucleotide gated currents were first reported in the sino-atrial node (SAN) in rats [9]. The current can only be slowly activated under hyperpolarization of the cell membrane. Therefore it was named as I_f or I_q due to this unique property ("funny" and "queer"). Later, HCN current was also found widely in neurons, where it was called I_h.

HCN channels belong to the super-family of voltage gated potassium channel. Similar to Shaker-type potassium channels, functional HCN channels are assembled with four subunits of the same subtypes (homotetramer) or different subtypes (heterotetromer) [10–13]. Each subunit consists of six transmembrane α-helix domains (S1–S6) (Fig. 1a). Between S6 and the C terminal is a cyclic-nucleotide binding domain (CNBD). The sequence of CNBD domain is highly homologous to that of cyclic-nucleotide gated channels (CNG) [14, 15], which can modulate the activation of HCN channels by directly binding with cAMP. Most of the transmembrane domains, including S4 voltage-sensor, pore region and CNBD domain, are highly conserved (80%–90%) among the four subtypes of HCN channels, while the N- and C-terminals are more variable [15, 16].

Typically, HCN channels can be activated when membrane potential is hyperpolarized negative to about $-50 \sim -60$ mV, suggesting the partial activation of HCN channels under resting potential. The channels are permeable to both Na^+ and K^+; the reversal potential of I_h is about $-20 \sim -40$ mV. Opened channels allow more influx of Na^+ and less efflux of K^+ with a net inward current, producing the depolarizing "sag" of membrane potential. Among four subtypes, HCN1 shows fastest activation and largest current compared to the other subtypes [17, 18]. The activation of HCN channels can be facilitated by the direct binding of cAMP or cGMP to the CNBD domain, which manifests as right (depolarization) shift of the activation curve (Fig. 1d). Therefore, any neurotransmitters that can up- or down-regulate the level of cAMP or cGMP are able to modulate the activation of HCN channels, such as β-adrenalin agonist [19], 5-HT [19], Ach [20], NO [21]. Interestingly, the extent of modulation by cAMP differs among the four subtypes. HCN4 can be modulated most significantly, while HCN1 is almost unaffected [22]. Similar regulation takes place when

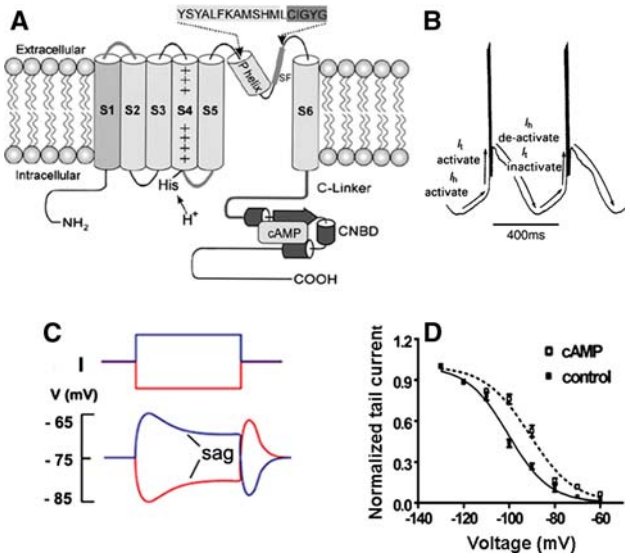

Fig. 1 Structure and primary physiological functions of HCN channels. (**a**) A schematic drawing of the structure of single HCN channel unit (modified from [16] with permission). (**b**) I_h, along with I_T, serves as the pacemaker currents in the SAN cells and thalamic delay neurons (modified from [22]). (**c**) In neurons with no automatic activity, I_h contributes to the maintenance of membrane resting potential. It opens when membrane potential is hyperpolarized and the inward currents draw the membrane potential toward resting condition. It closes when membrane potential is depolarized, the decreased inward currents prevent excess depolarization (modified from [22]). (**d**) Voltage dependence of I_h activation in DRG neurons and the effects of 100 μM cAMP. The dependence was shifted toward more depolarization potential in the presence of cAMP. Curves were fitted with Boltzmann equation (our unpublished data). (Fig. 1b, c were reprinted with permission, from the Annual Review of Physiology, Volume 65 ©2003 by Annual Reviews, http://www.annualreviews.org)

phosphoinositide, such as PIP2, directly binds to HCN channels at some presumed "PIP domain". But this modulation is not subtype-specific [23]. In the study of HCN current, Cs^+ is a useful blocker because of its rapid onset and the reversibility after washout; the drawback is that it cannot differentiate I_h from some potassium channels. In comparison, another blocker, ZD7288, shows much higher specificity, thus is most widely used.

The most typical function of I_h is its involvement in the pacemaking activity of cardiac SAN cells and a variety of neurons that have spontaneous firing or display sub-threshold membrane oscillation, such as thalamocortical relay neurons [19, 24, 25], hippocampal stratum oriens-alveus interneurons [26], and injured dorsal root ganglion neurons [27, 28] (Fig. 1b). Accordingly, I_h (I_f) is also called pacemaker currents. Notably, I_h is necessary but not sufficient for pacemaking in these neurons. In general, activation of I_h during the after hyperpolarization phase of the spike trajectory drives the membrane potential toward the firing threshold; T type calcium channels, which carry more rapid depolarization currents, will elicit a spike

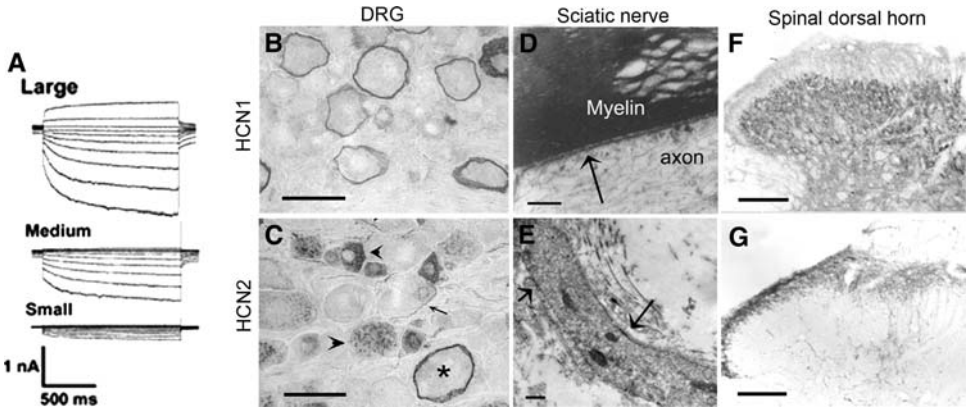

Fig. 2 Distribution of HCN channels and I_h in pain pathways. (**a**) Typical samples of I_h current in different DRG neurons [37]. The amplitude of I_h is highest in large DRG neurons; relatively smaller current can be seen in medium and small-sized neurons. (**b**, **c**) HCN1 and HCN2 in DRG [51]. HCN1 is predominantly localized on the membranes of large and medium-sized DRG neurons. HCN2-ir can be detected on the membrane of large neurons (asterisk), cytoplasm of small and medium-sized neurons (arrow), and occasionally in the axons of small neurons (arrow head). Scale bar: 50 μm. (**d**, **e**) HCN1 and HCN2 in the sciatic nerve fibers [51]. HCN1 positive staining can be detected on the axolemma of myelinated axons (arrow head). HCN2 positive staining can be detected on the axolemma of unmyelinated axons (arrow head). Scale bar: 0.5 μm. (**f**, **g**) HCN1 and HCN2 in the spinal dorsal horn (unpublished data). HCN1 is mainly expressed in the lamina III to IV. HCN2 is mainly expressed in lamina I. Scale bar: 100 μm. Figure 2a was reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

subsequently [22]. For those neurons with no automatic activities, I_h is considered to be crucial in determining their resting and passive cable properties [22]. On one hand, I_h helps maintain and limit the resting potentials at a certain level (Fig. 1c). On the other hand, the partial opening of HCN channels at resting potential contributes to the resting membrane conductance, thereby set the input resistance (R_{in}), membrane time constant and length constant. This enables I_h to modulate the response of excitable neurons to outside stimuli. For example, the presence of I_h decreases R_{in} and dampens the response of neurons to certain depolarization input; in reverse, the absence of I_h increases R_{in} and augments neuronal response to the same input. Such modulation happens in some postsynaptic neurons and was considered to serve as a homeostatic mechanism in response to changed synaptic activity [29]. In addition, I_h is also detected in presynaptic sites and has a role in synaptic transmission through regulating presynaptic release of neurotransmitters [30, 31]. However, this function remains controversial due to some nonspecific effects of ZD7288 (see below).

Distribution of HCN Channels in the Pathway of Pain Transmission and the Their Physiological Functions

HCN channels have been found to be expressed throughout the cardiac vascular systems, sensory and motor nervous systems [17, 32, 33]. In the past decades, much attention has been paid to their role in the sensory nervous systems, e.g. vision [34]. Shortly after I_h was identified in the rabbit SAN cells, Mayer and Westbrook, for the first time, reported the existence of I_h in the mouse embryo DRG neurons in early 1980s [35]. Years later, it was found that the distribution of I_h varied among different types of primary afferent neurons [27, 36–42] (Fig. 2a). I_h shows higher current density and amplitude, faster activation, and appears more frequently in large and medium-sized (A type) primary afferent neurons than that in small (C type) neurons. It was also found that I_h was mainly expressed in TTX sensitive A type neurons in contrast to the much smaller magnitude in capsaicin sensitive C type neurons [36]. It turned out that those I_h-riched large neurons had shorter duration action potential and time dependent rectification under current clamp conditions, while the neurons with little or no I_h showed longer duration action potential and no time-dependent rectification [40]. I_h is thus believed to mainly contribute to determine the resting potential of A type primary afferent neurons in normal conditions.

After the four cDNAs encoding HCN channels were cloned, the distributions of HCN channels in primary afferent neurons have been investigated intensively [27, 37, 43, 44]. In DRG, HCN1 is the most abundant subtype and can be detected in virtually all of large and medium-sized DRG neurons and in a few small ones. It is mainly localized on the membrane of cell soma (Fig. 2b). The expression of HCN2 is somewhat lower than that of HCN1 and can be detected in about half of all types of neurons. Interestingly, HCN2 is mainly expressed on the membrane in large neurons and sometimes colocalized with HCN1; while in small and medium-sized neurons, it is localized intracellularly and is colocalized with CGRP, the marker of peptidergic nociceptive neurons (Fig. 2c). The expression levels of HCN3/4 seem much lower than that of HCN1/2.

Considering the highest current amplitude and the fastest activation of HCN1 among the four subtypes, the expression patterns of HCN subtypes in DRG are properly matched with the biophysical characteristics of I_h in different sized DRG neurons.

HCN channels are also expressed in the spinal dorsal horn [37, 44, 45] (Fig. 2f, g). HCN1 can be detected primarily in myelinated axon terminals in lamina I and III to IV, consistent with their expression pattern in DRG. HCN2, on the other hand, was located in the central terminals of nonmyelinated peptidergic afferents in lamina I to out part of layer II (IIo). Further work showed that HCN2 positive terminals were predominantly apposed to excitatory interneurons in the spinal dorsal horn [37, 44, 45]. HCN3 can be found in the neurons across the spinal dorsal horn, while HCN4 positive staining can be found in both neurons and nerve fibers throughout the spinal dorsal horn (unpublished data).

So far, little attention has been paid to the expression of HCN channels in the peripheral sensory terminals. Recently, Luo et al. investigated the distribution of HCN subtypes in the glabrous skin of naïve rats' hind paw plantar [46]. They showed that HCN1 could be detected in the Meissner's corpuscle in dermal papillae and Merkel cells in epidermal layer. Meissner's corpuscles are innervated by both myelinated and unmyelinated afferents, and considered to be sensitive to noxious stimuli in addition to their low threshold mechano-sensitivity. Merkel cells are mechanoreceptors innervated mainly by myelinated afferents [47]. Expression of HCN2 is similar to that of HCN1 in Meissner's corpuscle. We additionally found abundant expression of HCN2 in the CGRP positive free nerve endings in the epidermal layer (unpublished data).

I_h is also detectable in the myelinated and nonmyelinated axons of mammalian peripheral and central nervous systems when the axons are hyperpolarized by applying tetanic stimulation [48, 49]. Its effect on excitability is more prominent in sensory than in motor axons [50]. In our recent study, we found very little HCN1-*ir* signals and relative more HCN2-*ir* with linear staining pattern with immunohistochemical staining. Their subcellular distributions were also observed by immuno-electromicroscopy approach (Fig. 2d, e). We found that HCN1 positive staining can be clearly found along the axolemma of myelinated axons, HCN2-*ir* was seen along the membrane of unmyelinated axons in a segmental pattern. Nevertheless, the expression of HCN channels in the axon is indeed more limited than that in DRG and central nervous systems [51].

Although much work has highlighted the involvement of cerebral I_h in a variety of physiological and pathological conditions, such as slow wave sleep and epilepsy [22], so far there is little work concerning about its expression and function in the supra-spinal pain pathways. Recent studies showed the existence of I_h in the ascending and descending regulation pathways of pain transmission, including μ-receptor containing neurons in dorsal raphe nucleus [52], and GABAergic neurons in raphe nucleus magnus and periaqueductal gray (PAG) [53]. The regulation of I_h by cAMP in these areas was found to participate in the withdrawal pain sensation after chronic morphine treatment. Additionally, both I_h and HCN-*ir* were found in the neurons in trigeminal nuclei and were believed to contribute to the trigeminal neuralgia [54].

Role of I_h in Neuropathic Pain

Recently, a series of behavioral studies on a variety of animal models consistently showed the contribution of I_h to neuropathic pain. When ZD7288 was given to the neuropathic pain models either intraperitoneally [27], or intrathecally [55], or perineuronally to the injured sciatic nerve [51], or intraplantarly to the injured hind paw [46], or topically to the injured DRG (our unpublished data), significant analgesic effects were observed with no disturbance in motor function (Fig. 3b–d). Besides, several clinically used analgesics were suggested to function partially through the inhibition of I_h, such as loperamide [56], propofol [57] and clonidine [58]. In this section, we attempt to discuss extensively about potential analgesic mechanisms of I_h.

Pacemaker Currents in Spontaneous Ectopic Discharges

Ectopic discharges were initially recorded in the sciatic nerve neuroma and later in all other neuropathic pain models, and were suggested to be involved in the development of mechanical allodynia and spontaneous pain [59–63]. This kind of spontaneous activity was generated from injured DRG neuronal soma or axons, as well as adjacent uninjured nerves. DRG neurons and their axons barely fire action potential on their owns under normal condition, therefore, those activities are called "ectopic discharges". There are three typical patterns of ectopic discharges, tonic (regular), burst (on and off), and irregular discharges; some of them are initiated and companied by subthreshold oscillation, some are not [64, 65]. The spontaneous property of ectopic discharges is similar to that of SAN cells, thalamic relay interneurons and other neurons with rhythmic automatic activity, thus I_h likely has a role in ectopic discharges.

There are three pieces of evidence supporting the role of I_h in ectopic dischares. *First*, ectopic discharges are essentially generated from large and medium-sized DRG

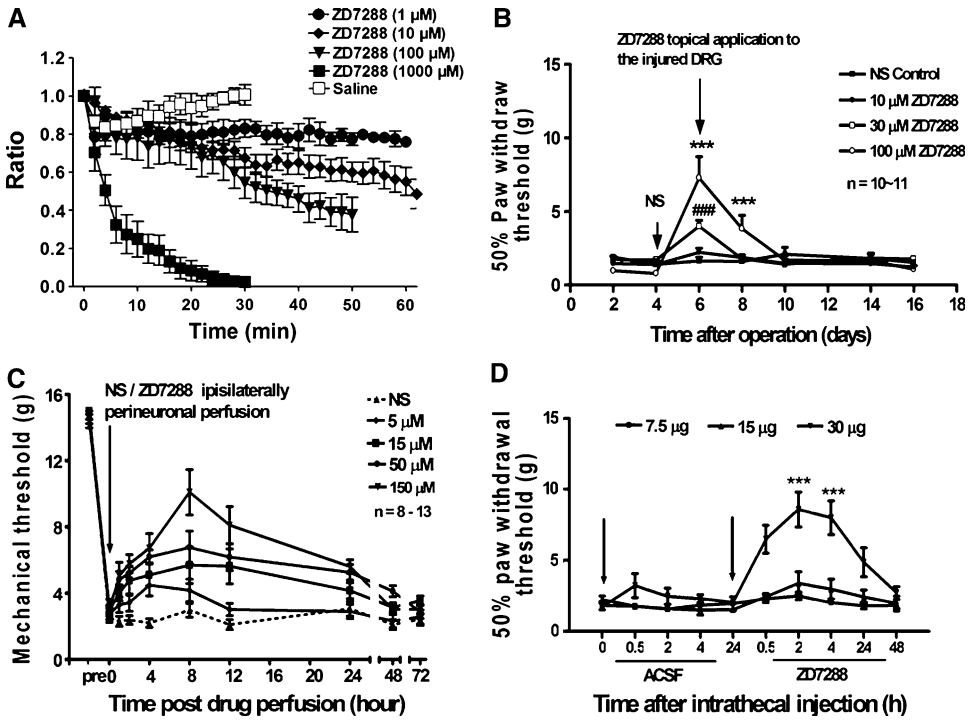

Fig. 3 HCN channels contribute to the development of neuropathic pain. (**a**) In SNL model of rats, topical application of ZD7288 to the injured DRG significantly suppresses ectopic discharges in a concentration-dependent manner [28]. (**b**) Topically application of ZD7288 to the injured DRG inhibits the mechanical allodynia in SNL rats (unpublished data). (**c**) Perineuronal perfusion of ZD7288 markedly alleviates mechanical allodynia in CCI rats [51]. (**d**) Intrathecal injection of ZD7288 significantly reverses mechanical allodynia in SNL rats

neurons in neuropathic rats of the spinal nerve ligation (SNL) [60], the sciatic nerve chronic constriction injury (CCI) [66], the DRG chronic compression (CCD) [67] and the early stage of neuroma [63], consistent with the fact that I_h is present primarily in these large and medium-sized neurons. *Second*, an obvious upregulation of I_h density and activation rate were observed in large and/or medium-sized DRG neurons 1–3 weeks after SNL injury [27] and within 1 week after CCD injury [68]. Same changes were also found in large and medium-sized TRG neurons 3 days after CCI of infraorbital nerve [69]. In sciatic nerve CCI model, we detected significantly increased expression of both HCN1 and HCN2 at the site of injury [51]. However, I_h was decreased in the DRG neurons 2–7 weeks after transection of sciatic nerves in neuroma model [70]. We also observed a remarkable reduction of both I_h and HCN proteins 2–5 weeks after SNL (unpublished observation). *Third*, low concentration of ZD7288 significantly suppresses ectopic discharges generated from either injured DRG neuronal soma or axons in both in vivo and in vitro studies [27, 28, 51, 71] (Fig. 3a). The inhibition efficiency is much higher in large neurons than that in small neurons [27].

How does I_h participate in the generation of ectopic discharges? It was proposed that pacemaking property of I_h might contribute. However, if this is the case, it would be hard to explain the slower activation rate of HCN channels (hundreds of milliseconds around resting potential) versus the high frequency of some of the ectopic discharges (up to 100 Hz) [5]. Although Yao et al. detected that the time constant of fast activation of I_h was significantly increased (to near 200 ms under −70 mv) in the dissociated DRG neurons in the CCD model [68], it is still unlikely that I_h will be able to trigger such fast firing. Chaplan et al. found no change of fast activation constant in SNL models under −140 mV in DRG neurons. Aβ subunit for HCN channels, Mink-related peptide (MiRP1), was found recently to be able to accelerate the activation of HCN channels when it was co-expressed with HCN1 or HCN2 in *Xenopus* oocytes [72]. Besides, in vivo and in vitro studies showed that the activation rate of HCN channels, as well as that of other ion channels, could be slowed down by the inhibition of tyrosine protein kinase [73]. It was also reported that *N*-glycosylation of potassium channels and sodium channels could increase their activation kinetics [74, 75]. Since functional HCN channels are also *N*-glycosylated proteins [76], similar modification could take place on them. Therefore, it is possible that the change of channel assembly among HCN subunits and non-HCN subunits, or the regulation by protein kinases and glycosylation after nerve injury will speed the activation of HCN channels. These changes together likely enable I_h to contribute to the rapid ectopic discharges.

A number of other unsolved problems are yet to be investigated. For example, it is hard to reconcile the increased density of I_h with paradoxically decreased HCN1/2 mRNAs and proteins in DRG 1–3 weeks after SNL injury [27]. Although it is likely due to the change of subunit polymerization or regulation of other accessory proteins, there is no direct evidence at this point. Besides,

recent studies have indicated the critical role of ectopic discharges in the trigger of central sensitization and neuropathic pain behaviors in the early stage (within 1 week) after peripheral nerve injury [77, 78]. Therefore, the change of HCN channels and I_h in the early stage and their contribution to trigger and maintain neuropathic pain are worthy further investigation.

Subthreshold membrane oscillation (STMO) is considered to be a trigger of ectopic discharges [64, 65, 79, 80]. It has been reported that inhibition of I_h could suppress STMO in a variety of central neurons, such as thalamic relay neurons and inferior olive neurons [25, 81, 82], I_h hereby contributes to the development of STMO in these neurons. However, to our knowledge, there are no studies investigating this issue in DRG neurons till now. Some inconsistency indeed exists between the characteristics of I_h and that of STMO generated from injured DRG neurons [5–7]. One is that much higher frequency of STMO in DRG neurons is in contrast to the slow activation rate of I_h. Similar to what we discussed above about the involvement of I_h in some fast firing ectopic discharges, it is likely that the activation rate of I_h could be regulated by some post-translation modulations. Second, STMO sustains under resting potential in which very little HCN channels are activated, yet this discrepancy could be partly explained by the finding of increased I_h density as well as the shift of its activation curve toward depolarization potential after nerve injury [27, 28]. The most prominent contradiction lies in their opposite voltage sensitivity, i.e., depolarization-facilitation for STMO [65] versus hyperpolarization-activation for I_h. Although intracellular recording in some central neurons confirmed that inhibition of I_h suppressed STMO by affecting the after-hyperpolarization [25, 81, 82], so far there is no evidence to exclude the possibility that hyperpolarization of membrane potential, which can be induced by inhibition of I_h, per se participated in the suppression of STMO. Further work needs to be done to clarify the mechanisms involved in the regulation of I_h in STMO of injured DRG neurons.

Involvement in the Sensitization of Nociceptors

In addition to the abnormal discharges of injured neurons, adjacent uninjured afferents also undergo hyperexcitability after peripheral nerve injury. A most remarkable change is the sensitization of peripheral nociceptors which characterized by the decreased threshold for activation, increased or exaggerated response to noxious stimuli, as well as spontaneous activity [83]. A number of ion channels, such as $Na_v1.8$, T-type calcium channels, TRPV1 and acid sensitive ion channels [4], are suggested to contribute to the sensitization of peripheral nociceptors.

Up to date, there are only a few studies addressing the functional role of peripheral HCN channels. A behavioral test recently demonstrated an obvious but insignificant reversal of mechanical allodynia by intraplantar injection of 100 μM ZD7288 to the partial sciatic nerve injury rats [84]. Recently, another study showed that HCN channels were expressed in the peripheral receptors [46] as mentioned above. In addition, they also found that the mechanical allodynia of SNL rats and spontaneous pain of mild thermal injury (MIT) rats were significantly alleviated by intraplantar application of 30 mM ZD7288, while the thermal hyperalgesia of MIT rats was unaffected. These findings indicate that HCN channels in the peripheral terminals are pro-nociceptive in the neuropathic pain models. However, so far there is no report examining the change of expression or biophysical properties of HCN channels in nociceptors after peripheral nerve injury. Two points should be noted with regard to these studies. First, in both studies, the analgesic effect of ZD7288 was transient and completely disappeared within 1 h after administration. However, it is well known that the inhibition of HCN channels by ZD7288 is irreversible. In agreement with that, we also found that suppression of ectopic discharges by ZD7288 lasted throughout our experiment (at least 1 h) without recovery in both in vivo and ex vivo recording. Also, analgesic effect of ZD7288 by systemic or perineuronal application in neuropathic pain rats lasted for nearly 24 h [27, 51]. This discrepancy could be due to the different metabolism mechanisms of ZD7288 in different systems. Second, the concentration of ZD7288 used in one study was 30 mM, which is hundreds of times higher than the effective concentration used in previous studies of I_h. Therefore, we can hardly exclude its nonspecific effects.

On the other hand, an interesting study regarding the role of HCN channels in the aortic baroreceptors might provide us some hint about its potential role in peripheral nociceptors [43]. Like other sensors, baroreceptors are activated by certain stimuli, for example, pressure. It was found that inhibition of I_h in baroreceptors upregulated the excitability of baroreceptors through increasing the membrane input resistance, leading to a decreased pressure threshold for activation and increased discharges in response to supra-threshold pressure. These characteristics are reminiscent of sensitized nociceptors after peripheral nerve injury. In this regard, peripheral I_h likely has an anti-nociceptive role, which still remains an open question.

Regulator in the Axonal Conduction

I_h is expressed in both myelinated and unmyelinated axons. It was found that activation of I_h inhibited the membrane hyperpolarization and the slowing of axonal conduction induced by tetanic stimulation with low frequency in the

ex vivo study (activity-dependent slowing of conduction) [85, 86]. Recently, we also detected the relatively lower expression of HCN1 and HCN2 channels on the axolemma of axons. Previous work found that clonidine was able to strengthen and prolong the peripheral nerve blocking effect of local analgesics through inhibiting axonal I_h [58, 87]. Consistent with the role of I_h in axonal conduction, Dalle et al. reported that perineuronal administration of ZD7288 significantly alleviated the mechanical allodynia of partial sciatic nerve injury rats [84, 88]. However, other studies indicated that ZD7288 was not able to block axonal conduction [27, 51]. It is likely, in this case, that the analgesic effect of ZD7288 and clonidine is partially due to the inhibition of ectopic discharges generated from the injury sciatic nerves rather than their effects on axonal conduction. In agreement with this idea, we recently found that perineuronal ZD7288 perfusion can markedly relieve mechanical allodynia via inhibiting ectoptic discharges in CCI model [51]. The properties of activity-dependent slowing of conduction in uninjured sciatic nerves were found to be increased in SNL models [87], presumably due to the decreased expression of I_h in the uninjured axons. However, whether and how this change contributes to the development of neuropathic pain behaviors is yet to be studied.

Role in the Synaptic Transmission in Spinal Dorsal Horn

The strengthened nociceptive synaptic transmission in the spinal dorsal horn, which is part of the central sensitization, is another critical inducer of neuropathic pain behaviors [89]. Increased release of exciting neurotransmitters, such as substance P (SP) and glutamate, from the nociceptive central terminals contributes a great deal to neuropathic pain. As mentioned above, HCN channels are expressed in the nociceptive and mechano-sensitive central terminals in the spinal dorsal horn. Moreover, HCN2-positive terminals also contain SP and glutamate. They formed synaptic connections with excitatory interneurons. These kinds of distribution likely enable HCN channels to participate in the regulation of presynaptic release of neurotransmitters and subsequently to regulate the excitability of interneurons in the spinal dorsal horn. Consistent with this idea, the in vitro recording on the spinal cord slices showed that the number of monosynaptic excitatory postsynaptic potential (mEPSP) induced by electrical stimulation of primary afferents was slightly reduced by ZD7288, suggesting the inhibition of synaptic transmission by ZD7288 [45]. Accordingly, we found that intrathecal administration of ZD7288 reversed mechanical allodynia significantly [55]. However, the role of I_h in the synaptic transmission was questioned because of nonspecific effects of ZD7288 on AMPA and NMDA receptors, which will be discussed below.

I_h Blockers and Transgenic Approaches

So far a number of HCN blockers have been discovered, including Cs^+, ZD7288, alinidine (ST-567), zatabradine (UL-FS49), DK-AH 268 and ivabradine (S16257) [22]. Inhibition of I_h by these blockers can reduce the rate of heart beat, hence I_h blockers are also called bradycardics [90]. ZD7288 is the most widely used in the study of I_h due to its high specificity and commercial availability. Therefore, most of our knowledge about the functions of I_h, including its role in pain, has been obtained by using ZD7288.

However, some of these findings are recently questioned as a result of the discovery of the nonspecific effects of ZD7288. For example, T-type calcium channels were found to be inhibited by relatively higher concentration of ZD7288 in the mouse spermatogenic cells [91]. It was considered that I_h and I_T cooperated in the pacemaking process in both SAN cells and thalamic relay neurons. It would be very hard to differentiate the role of these two channels by ZD7288. However, there are two clues that might be helpful in clarifying this issue. First, the inhibition of I_h by ZD7288 is much more effective than that of T-type calcium channels (IC_{50} was 2 μm and 100 μm for inhibition of I_h and I_T respectively). It is reasonable to assume that ZD7288 will preferentially inhibit I_h rather than I_T. Second, the effect of ZD7288 on I_h is ir-reversible, in contrast, I_T showed partial recovery after washout. In view of the sustained suppression of ectopic discharges by low dose of ZD7288, it is more likely that ZD7288 targets at I_h.

Other nonspecific target of ZD7288 involves the controversy surrounding whether I_h participates in the regulation of synaptic transmission. It was found that the inhibition of LTP or EPSPs by low dose of ZD7288 (20–50 μM) was likely dependent on the inhibition of postsynaptic NMDA or AMDA receptors [92] or other presynatpic mechanisms [93]. These findings challenged the proposed role of I_h in the nociceptive synaptic transmission of spinal dorsal horn in the development of neuropathic pain. Thus it remains unclear whether I_h was convincingly involved in the alleviation of mechanical allodynia when ZD7288 was applied intraperitoneally or intrathecally.

Ivabradine, known as Procarralan commercially, is considered to be the most specific blocker of I_h without effect on T-type, L-type calcium channels and delayed outward potassium channels [90]. Recently it was approved by European Medicines Evaluation Agency as a new treatment drug for patients with chronic stable angina pectoris. This drug can slow the heart rate by inhibiting I_h.

It is of great interest to confirm the behavioral and electrophysiological studies of neuropathic pain with this drug.

Very recently, HCN1, 2 and 4 transgenic mice have been successfully established and widely used in understanding various physiological and pathological functions of I_h in cardiovascular and nervous systems. HCN knockout mice consistently exhibit the disturbed heart beat, recapitulating the pharmacological results obtained in the past decades [94, 95]. Interestingly, over-expression of HCN2 and 4 had been found to accelerate the heart rates [96]. These studies strongly suggest the pacemaking function of I_h in SAN and central neurons. However, their applications in the study of pain are still in absence. It would be interesting to examine if there are any alterations of ectopic discharges and neuropathic pain behaviors on these transgenic mice.

Conclusions and Future Directions

In the past decade, following the cloning of HCN channels, rapid progress has been made in the study of the structure, biophysical properties as well as functions of HCN channels. I_h shows broad physiological functions and participates critically in many pathological conditions. While the role of I_h in pain has also attracted much attention recently, a number of unsolved problems remain to be explored. For example, the exact biophysical mechanisms of I_h in ectopic discharges is unclear. The role of I_h in spinal central sensitization is yet to be defined. Nevertheless, with the development of more specific blockers and transgenic methods, more progresses will be made in understanding mechanisms concerning how I_h participates in the development of neuropathic pain, and then hopefully, in the exploitation of new clinical analgesics. However, the broad distribution of HCN channels throughout peripheral and central nervous system inevitably brings problems to the clinical application of HCN blockers. Mild vision disorder, for example, was found occasionally during the treatment of angina patients with ivabradine [90]. The application of HCN blockers as analgesic will definitely face the same question when they are taken orally or in vein. However, some remarkable features of the involvement of HCN channels in neuropathic pain are likely to provide some clues in designing drugs. For instance, HCN channels mainly function in the peripheral pain pathways especially in the ectopic discharges. Besides, the predominant subtypes in pain pathways are HCN1 followed by HCN2, contrast to HCN4 in SAN cells [16]. Hereby, subtype specific, blood brain barrier impermeable synthetic agents will be much more effective in analgesia and accompanied by fewer side effects ideally. Alternatively, local application might be a feasible approach as well. We have witnessed the approval of ivabradine in the clinical treatment of angina. There is no reason to doubt that, although there is a long way ahead, I_h could also become a valuable target in the clinical treatment of chronic pain.

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