CURRENT UNDERSTANDING OF HYPERPOLARIZATION-ACTIVATED AND CYCLIC NUCLEOTIDE-GATED (HCN) CHANNELS: A REVIEW

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ABSTRACT

Hyperpolarization-activated and Cyclic Nucleotide-gated (HCN) channels with significant physiological role are found to be present in heart and brain. These channels have got four subtypes encoded by four different genes. They play significant role in maintainance of cardiac rhythm and neuronal excitability with definite but partially defined role in development of arrhythmia, epilepsy and neuropathic pain. These are also known as pace maker channels. In present review we will mainly discuss electrophysiology of neurons and SA node in special reference to HCN channel. In the heart mainly HCN4 subtype is present while in brain all four isotype have been found where they are involved in numerous neuronal functions i.e. dendrite c integration, memory, thalamo-cortical rhythm etc. Further in review we will also discuss in brief the physiology and uniqueness of this ion channel and disorders especially epilepsy, neuropathic pain and cardiac arrhythmia, due to the malfunctioning of these ion channels. The newer possibilities of modifying this ion channel along with the drugs acting will also be discussed.

Keywords: HCN ion channels, Arrythmia, Epilepsy, Neuropathic Pain.

INTRODUCTION

Rhythm, classically defined as any motion characterized by regular recurrence or pattern in time which can be applied to numerous cyclical natural phenomena with periodicity. The rhythmic character can be observed in numerous natural systems including human body. There are various activities which require considerable synchronizing activities e.g. Pace maker, central pattern generator, motor co-ordination, perception etc. Out of these numerous activities we will mainly focus on activities associated with heart and brain. As these actions are vital to various physiological activities, there malfunctioning can result in disease states. The variety of factors play critical role in maintaining the rhythmicity in sinoatrial (SA) node as well as neuronal synchronization. There are numerous ion channels are involved in maintaining this normal physiological rhythm of SA node including hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. HCN channels initially referred to as pacemaker channels[1], supposed to have a key role in the control of heart rate and neuronal excitability as they help to generate rhythmic activity within groups of heart and brain cells. These channels belong to the family of cyclic nucleotide regulated channels and function as nonselective ligand-gated cation channels. Cyclic nucleotide regulated channels also include cyclic nucleotide-gated (CNG) channels. The activation of CNG channel is mediated by direct binding of cGMP or cAMP to the channel itself[2,3]. CNG channels are permeable to both K+ and Na + ions. Since the CNG channels are activated by second messanger they offer a certain mechanism through which the concentration of second messanger can directly alter the membrane excitability. In the manner similar to CNG channels the HCN channels are also activated by second messanger. The difference between these two lies in the fact that CNG channels are independent of voltage while HCN channels are voltage dependent[4]. These are some of the distinguishing features of HCN channels from CNG. HCN channels comprise four subtypes which are encoded by four separate genes i.e. HCN1-45. Functionally, they differ from each other in terms of time

constant of activation (HCN1 being the fastest and HCN4 slowest)[6] and sensitivity to cAMP. Both cyclic nucleotides i.e. cAMP and cGMP are full agonists of these channels at saturating concentrations[7]. These channels are found in various sites in human body but in this review we will focus mainly on their distribution in brain and heart. In the central nervous system HCN1 is expressed in neocortex, hippocampus, brainstem, cerebellar cortex, spinal cord[8-12]. HCN2 is mainly present in thalamus and brain stem [9-11]. HCN3 is present in olfactory bulb[9-10]. HCN4 is usually present in thalamic nuclei and olfactory bulb[9-11]. In the heart every subtype have been found and expression of particular subtype varies in between different regions of heart. HCN4 is the major subtype present in SA node[13], AV node[14] and purkinje fibres[15].

Unique Features of HCN Channels

The HCN channels possess remarkably different properties and different kinetics from other voltage gated ion channels. In the above section we have discussed some of the properties. Further in continuation although these channels are structurally similar to K+ ion channels they show relatively less selectivity for K+ and they are better permeable for Na+ [16,17]. In contrast to other voltage gated ion channels, they are partially open at resting membrane potential and their activation increases with hyperpolarization, hence we can conclude that resting state of cells activate them while depolarizing state turn them off. It seems it is the fine balance between active and relative inactive state which is exquisitely sensitive to the minor changes in membrane potential may provide stability to neuronal and cardiac cells may also contribute to rhythmicity. As these channels show constitutive activity i.e. they are active even in resting condition, this itself signifies their important role in membrane physiology. As quoted earlier in the article these channels are also modulated by second messanger e.g. cAMP, this is another circumstantial evidence that these channels are tuned in such a manner so as to provide stability to the cell. From above discussion we can infer that HCN channels are nature's mechanism to prevent over stimulation of cell. With this background we now will discuss the physiological role of HCN in heart and brain.

Role of HCN Channels in Cardiovascular Physiology

SA node has got prominent role in generation of heart beat and maintaining the automaticity of heart beat because of generation of pacemaker potential defined by diastolic depolarization in the voltage range of -65 to -45mV. It is the diastolic depolarization which causes membrane potential of SA node back to threshold, hence maintaining the automaticity. The current produced by HCN channel is known as Ih, Iq or If where the subscript stands for hyperpolarization, queer and funny respectively and is supposed to play prominent role in diastolic depolarization[18] along with the other ionic currents. Other factors responsible for diastolic depolarization are sarcoplamic release of calcium through ryanodine receptors[19], the sustained inward current Ist[20], T-type Ca++ current (ICa,T)[21], and L-type Ca++ current (ICa,L)[22].

If currents are inward current activated on hyperpolarization to the diastolic range of voltages, have characteristics suitable for generating automaticity and for modulating spontaneous heart rate. As If currents are controlled by intracellular cAMP hence they are activated and inhibited by adrenergic and muscarinic M2 receptor stimulation respectively, which represents a basic mechanism through which effects of autonomic nervous system on the heart are mediated.

Since HCN4 is major subtype present in SA node so deletion of its gene in animal model certainly will have detrimental effects. There are definite evidence from different studies that mouse embryos which were lacking functional *HCN4* failed to develop during the embryonic period and died in utero[23,24]. On the other hand their role in adult mice is not very clear as they are rarely affected24, although the intensity of If declined but the SA node activity didn't affected much probably indicating presence of alternative mechanisms to overcome the defect. HCN2 is also present and their deletion can cause sinus dysrhythmia characterized by varying peak-peak intervals in the electrocardiogram [25]. Here it can be concluded that these channels are important for embryonic development and development of adult pace-making system.

Role of HCN Channels in Neuronal Physiology

As mentoined earlier these currents have certain unique features which are important for electrical responsiveness of cell. These channels are partially active at rest, activated at hyperpolarization and deactivated at depolarization, with the property of negative feedback which resists both inhibitory and excitatory stimuli which eventually helps cell to maintain its memebrane potential. These channels are constitutively active at resting membrane potential and act as "voltage clamp" [26] that tends to stabilize membrane potential. Ih have got definite role to play in the numerous basic and higher neuronal functions and we will discuss it one by one.

All four subtypes of HCN are found in brain [27]. The Ih limits hippocampal long term potentiation (LTP) indicated by enhancement of hippocampal learning and memory in the mice with selective deletion of HCN1 gene[28,29]. High HCN1 expression probably interferes with the generation of calcium spikes associated with LTP29 . On the contrary the HCN increased spatial working memory which is concerned with immediate conscious perceptual and linguistic processing which actively holds multiple pieces of transitory information in the mind, where they can be manipulated and it depends on prefrontal cortex. There is complex interaction between HCN and alpha-2 adrenergic receptor in prefrontal cortex which results in increased efficiency of synaptic input, and the activity of these neurons increases[30]. Complex motor learning and memory requiring repeated co-ordination which involve cerebellar circuit are badly influenced by deletion of HCN1 gene [31]. Most neurons have elaborately branching dendritic trees that receive thousands of synaptic inputs which may be excitatory or inhibitory and their processing requires dendritic integration. A single EPSP can not generate action potential hence it requires integration of multiple synaptic inputs spatially as well as temporally. There is a proven role of Ih in this complex process[32]. Most important physiologic action where Ih participate is thalamocortical (TC) rhythms. Oscillatory activity is an emerging property of the thalamocortical system which can be generated alone by a single neurons or in coordination with other neurons. The various types of oscillations within TC system are infra-slow oscillations, slow oscillations, delta oscillations, sleep spindle oscillations, beta-gamma oscillations, and ripples. Thalamocortical oscillations are produced in thalamocortical networks during sleep[33], sensory processing[34], and seizures[35]. Various models and hypotheses have been proposed to describe the contribution of Ih in TC oscillations and neuronal physiology, detailed discussion of which is not required in this particular article.

Disorders of HCN Channel Malfunctioning

In the previous section we have discussed the significant physiological role of HCN channel in neuronal and cardiac physiology and their malfunctioning can result in variety of disorders especially in brain. These channels are found to be involved in epilepsy[36], neuropathic pain[37] and cardiac arrhythmia25 and others. we will only focus on these three disorders.

Epilepsy is characterized by the occurrence of spontaneous seizures, i.e., bursts of synchronous firing of large populations of neurons. These are believed to result from abnormal regulation of neuronal excitability. It was year 2001 when HCN channels were implicated in epilepsy[38], since then role of HCN channels in epilepsy is a matter of debate. Epileptogenesis involves alterations in variety of ion channels which may be inherited or acquired. In the hippocampal neurons HCN channels are predominantly present in apical dendrites and play important role in dendritic integration and attenuates neuronal excitability 32. It has been shown in many studies that down regulation of HCN channel is associated with increase in neuronal excitability which contributes to epileptogenesis[39,40,41]. HCN2 null mice show absence epilepsy 25 while HCN1 null mice are more susceptible to kainic acid-induced seizures41. From animal studies we have came to know that HCN channels have got neuron modulating activity and their malfunctioning can result in epileptiform activity such correlation can also be observed in human beings[42,43] as evidenced by enhanced levels of HCN1 channel expression in dentate gyrus resected hippocampal nuclei in patients of temporal lobe epilepsy[44]. Since HCN channels work in sea-saw fashion with multiple functions of neuronal activity which are context and location dependent, their upregulation or downregulation both can result in disease state. Further the findings from animal models may not always be replicated in human also. We can c onclude that at least defect in HCN can provoke seizures and it will be oversimplification to explain mechanism of epileptogenesis in generalized model of epilepsy. To explain the exact mechanism we need to have specific models of epilepsy under different patho-physiologic settings.

Neuropathic pain is a symptom of diseases damaging nerves characterized by allodynia and dysesthesia and is believed to be due to abnormal activity of neurons of dorsal root ganglion[45]. The HCN channels have been found to be present in dorsal root ganglion[46] and Ih has been found to be involved in generation of ectopic discharges in DRG neurons[47]. There is evidence that after axonal injury the HCN channel contribute to abnormal activity in peripheral nerves48. HCN1 has been found to be up-regulated in case of damaged nerve evoking spontaneous pacemaker driven action potential [48]. Pain intensity mainly depends on the firing rate of neurons, and it can be modulated by HCN channels as HCN2 ion channel is now known to be a key downstream target in the cellular events associated with pain[49]. In animal model of acute and chronic pain it had been shown that Ih expression and modulation in the peripheral nervous system, including specialized sensory structures, may play a significant role in sensory processing and

contribute to spontaneous pain and tactile allodynia[50]. The peripheral block of HCN channel by ZD7288 produces antiallodynic effect which indicates these channels represent a novel target for nerve block treatment of postoperative and neuropathic pain[51]. From above discussion we can conclude that the expression of these channels is increased in nerve injury and their inhibition with the drugs is likely to benefit in the disease. In the future it is expected to play significant role in neuropathic pain.

HCN channels are found to be present in virtually every heart with HCN4 and HCN2 are major subtypes. Although these channels are not solely responsible for maintainance of rhythm but their genetic alteration or malfunctioning can result in arrhythmia. Molecular techniques have been tried to delineate the relationships between mutations of the HCN channels genes and alterations in the cardiac function. A missense mutation in hHCN4 gene induces the production of an isoform of the pacemaker channel with altered cAMP binding site this was supposed to be responsible for a familial form of an asymptomatic bradycardia[52]. There exists four different types of mutations associated with different types of arrhythmia in the human isoform of the HCN4 channel[53]. Increased ventricular expression of HCN channels possibly contributes to the ventricular arrhythmias[54]. Loss of function of HCN4 is associated with sinus nodal dysfunction and that a consequence of pacemaker channel abnormality might underlie clinical features of QT prolongation and polymorphic ventricular tachycardia developed under certain conditions[55]. HCN channels were suggested to contribute to the shortening of the action potential duration and the prevention of early after-depolarization in bradycardia which suggests that the HCN4 channel played a preventive role in triggering bradycardia-induced ventricular arrhythmias[56]. Considering the functional complexity of HCN channel in regulation of cardiac rhythm and their upregulation and downregulation both are associated with diseased cardiac states it is difficult to understand specific phenotypic aspect associated with the mutation. More studies are required to detect the exact role of HCN mutations in developing cardiac arrhythmias.

HCN Channels and Pharmacological Interventions

As stated previously numerous molecular, pharmacological and genetic (knockout and transgenic mice) studies have verified emerging role of these channels in epilepsy, neuropathic pain and cardiac arrythmia. As the very basic concept of drug development states that the target (here HCN channel) which we intend to exploit to alter the course of disease or to gain therapeutic benefit must have critical impact on the patho-physiology of disease. HCN channels *per se* represent valid targets for novel agents for different clinical conditions. These evidences will be discussed in following section.

Ivabradine was one of the initial molecules whose mechanism of action involved inhibition of HCN ion channel which was approved by the European Medicines Agency in 2005 to be used in symptomatic treatment of chronic stable angina pectoris. Heart rate reduction is a recognized therapeutic target in several cardiac conditions, including ischemic heart disease and heart failure. In ischemic heart disease high heart rate increases myocardial oxygen demand and reduces diastolic coronary blood flow which aggravates myocardial ischemia. Ivabradine by selectively inhibiting If acts as heart rate lowering agent which reduces heart rate without affecting the inotropism and avoiding other cardiac side effects. This selective blocking of If current will definitely have important therapeutic advantages. Ivabradine has use and rate-dependence action which depends on open close state of channel itself[57]. Since it affects predominantly HCN channels it doesn't alter other ionic currents. In the management of chronic stable angina Ivabradine has efficacy comparable to atenolol[58] and amlodipine[59]. In the BEAUTIFUL clinical trial of ivabradine on patients of coronary artery disease with ejection fraction <40% it was found that reduction in heart rate does not improve cardiac outcomes in

all patients with stable coronary artery disease and left-ventricular systolic dysfunction, but could be used to reduce the incidence of coronary artery disease outcomes in a subgroup of patients who have heart rates of 70 beats per minute or greater[60]. Another clinical trial SHIFT, involving patients with symptomatic heart failure and a left-ventricular ejection fraction of 35% or lower, were in sinus rhythm with heart rate 70 beats per min or higher supports the importance of heart-rate reduction with ivabradine for improvement of clinical outcomes in heart failure and confirm the important role of heart rate in the patho-physiology of this disorder[61]. Considering all these clinical evidences the utility of ivabradine seems to be limited to a restricted group of patients with a sinus rhythm with a heart rate ≥70 beats per minute. When given with metoprolol, ivabradine has been found to be effective in treatment of inappropriate sinus tachycardia[62] characterized by excessive resting heart rate or a disproportional increase in heart rate during exercise, who were refractory to monotherapy. So it is clear from the above discussion that ivabradine has got adjunctive role in the management of angina while of certain advantage in management of heart failure.

In the management of neuropathic pain HCN blocker can be of therapeutic advantage as their expression gets upregulated at dorsal root ganglion and ventral-lateral periaqudactal gray matter in case of neuronal injury[63]. Pharmacological blockade of HCN activity using the specific inhibitor ZD7288 reverses abnormal hypersensitivity to light touch and decreases the firing frequency of ectopic discharges without conduction blockade48. In the animal study it is shown that peripheral block of Ih produces an antiallodynic effect, which suggests that Ih channels represent a novel target for nerve block treatment of postoperative and neuropathic pain51. These data clearly demonstrate the beneficial effects in neuropathic pain and provide conclusive evidence for development of HCN channel blockers for the treatment of neuropathic pain[64].

"Epileptogenesis refers to the transformation of a normal neuronal network into one that is chronically hyperexcitable. There is often a delay of months to years between an initial CNS injury such as trauma, stroke, or infection and the first seizure. The injury appears to initiate a process that gradually lowers the seizure threshold in the affected region until a spontaneous seizure occurs. In many genetic and idiopathic forms of epilepsy, epileptogenesis is presumably determined by developmentally regulated events"[65]. Genetic studies suggest that the HCN channelopathy begins rapidly and persists after status epilepticus involving both transcriptional and non-transcriptional mechanisms, and may be an early contributor to epileptogenesis[66]. The most compelling experimental evidence linking reduced HCN function to epilepsy has been found using the HCN2 knockout mouse25. Since these are the significant genes for epilepsy, they can serve as drug target. Contrary to the conclusions drawn from animal studies HCN2 channel inhibitors can be epileptic because of the larger current (35% more) associated with mutant HCN2 which also shows gain of function[67]. Although these models have thrown light on mechanism of absence seizure, to apply these concepts on human beings is still requires human genetic studies. Although there is no currently approved anti-epileptic drug which acts selectively on HCN channels, but certain drugs have been shown to involve HCN channels in their mechanism. Lamotrigine and gabapentin have been shown to reduce action potential firing in dendrites, by increasing Ih, which may be one possible mechanism for their antiepileptic action[68,69]. Ketamaine has also been shown to act on HCN1 channel which provide a plausible neuronal mechanism for enhanced cortical synchronization during anesthetic induced hypnosis and suggest that HCN1 channels might contribute to other unexplained actions of ketamine[70]. HCN1 channel is also modulated by propofol[71,72] which may be responsible for some of the side effects of propofol, simultaneously provides opportunity to develop selective HCN1 channel inhibitor. To conclude, more human and animal studies are required to uncover precise

physiological role of Ih in epilepsy so that we can develop drugs which can be used to treat or atleast modify the course of illness.

CONCLUSION

Currently HCN channels considered as significant regulators of many biological processes not only in nervous system but in the heart as well. There is growing body of evidences implicating changes in HCN channel function as part of the aetio-pathogenesis of neuropathic pain, certain epilepsies and arrythmias. Especially HCN2 and HCN4 both are involved upto different extent in these three disorders. Since the exact and precise role of HCN channels in neuronal physiology is not yet described with certain gray areas, we first need to clarify those issues. Animal models and computer simulation techniques have certainly helped us in elaborating the physiology and genetic knockout studies have thrown light on channelopathies, but these findings needs to be verified for humans also. At present we have drug approved to be used in cardiac conditions, but no drug is available to be used in other disorders that we have discussed. Since many aspects of HCN channel still remains to be elaborated, it will be interesting to see how many of potential drug therapies suggested for disorders, replicate themselves into actual clinical practice.

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