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The funny current: Even funnier than 40 years ago. Uncanonical expression and roles of HCN/f channels all over the body

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ABSTRACT

Discovered some 40 years ago, the I_f current has since been known as the "pacemaker" current due to its role in the initiation and modulation of the heartbeat and of neuronal excitability. But this is not all, the funny current keeps entertaining the researchers; indeed, several data discovering novel and uncanonical roles of f/HCN channel are quickly accumulating. In the present review, we provide an overview of the expression and cellular functions of HCN/f channels in a variety of systems/organs, and particularly in sour taste transduction, hormones secretion, activation of astrocytes and microglia, inhibition of osteoclastogenesis, renal ammonium excretion, and peristalsis in the gastrointestinal and urine systems. We also analyzed the role of HCN channels in sustaining cellular respiration in mitochondria and their participation to mitophagy under specific conditions. The relevance of HCN currents in undifferentiated cells, and specifically in the control of stem cell cycle and in bioelectrical signals driving left/right asymmetry during zygote development, is also considered. Finally, we present novel data concerning the expression of HCN mRNA in human leukocytes.

We can thus conclude that the emerging evidence presented in this review clearly points to an increasing interest and importance of the "funny" current that goes beyond its role in cardiac sinoatrial and neuronal excitability regulation.

1. Introduction

Pioneering studies carried out in the mid-1950s proposed that the deactivation of a time dependent potassium conductance, dubbed IK2, could represent the electrical event responsible for the spontaneous depolarization observed in Purkinje Fibers (Noble, 1960; Weidmann, 1951). In the mid-late 1970s this view was challenged by studies on rabbit sinoatrial node (SAN) preparations/cells that pointed the attention to an inward current activated upon hyperpolarization and, due to this unusual property, named the "funny" current or I_f (Brown et al., 1979; Maylie et al., 1979; Noma and Irisawa, 1976; Seyama, 1976; Weiss et al., 1978). The mysterious dualism between the two processes: IK2 decay and the activating inward I_f, was finally solved by Dario DiFrancesco in his seminal study of 1981 where he reinterpreted the IK2 current (DiFrancesco, 1981) demonstrating that this phantom IK2 decay was nothing else than the real I_f current. Taken together these studies set the stage for the undisputable evidence that the I_f current provides a fundamental contribution to cardiac pacemaker generation and modulation. For a more comprehensive historical review of the "birth" of I_f see Carmeliet (2019) (Carmeliet, 2019), and DiFrancesco (2020) (DiFrancesco, 2020). Since its discovery, several elegant studies have thoroughly analyzed the kinetics and ionic features of I_f demonstrating that they were appropriate to support a fundamental role in the generation of the diastolic depolarization and modulation of heart rate. Due to this functional role associated with the intrinsic automaticity of pacemaker cells the funny current has

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since been known also as the "pacemaker" current. Soon after its discovery in the heart, the search for a similar current in neurons began and, by the end of the '80s, an almost identical current was identified and dubbed I_h (Pape and McCormick, 1989). With the introduction of molecular biology techniques in the ion channel field, the molecular correlates of the native f-current were identified and four Hyperpolarization-activated Cyclic Nucleotide-gated genes (HCN1-4) were cloned (Robinson and Siegelbaum, 2003). This step further made it possible to map the expression pattern of HCN channels in many cells and tissues. After 40 years, the role of this tiny conductance, responsible for initiating and modulating the heartbeat and neuronal excitability, is expanding far beyond these canonical functions. Indeed, data have accumulated demonstrating novel uncanonical roles of f/HCN channels. In the present review, we provide a comprehensive overview of the expression pattern HCN channels and, when available, their cellular functions in taste buds, glia, pituitary gland, blood cells, bones, smooth muscles, gastrointestinal system and urinary system. Moreover, HCN channel expression/role in the zygote, in stem cells and in mitochondria are also reviewed. To maintain the same nomenclature throughout this review, we will always refer to native currents as I_f or f-current independently from the type of cells/tissue in which it was studied.

2. HCN channels in oocyte maturation and fertilization

Understanding the physiological mechanisms controlling maturation and fertilization of mammalian oocytes is a fascinating task for its potential application in the treatment of infertility, and fine-tuning of both membrane resistance (Rm) and membrane voltage (Vm) is a critical step in these processes. Multiple studies have indeed confirmed the presence in ovarian and sperm cells of several ion channels and described their role in controlling the electrical properties of these cells (Agoston et al., 2004; Kunz et al, 2002, 2006; McCulloh and Levitan, 1987; Platano et al., 2005). In particular, Yeh and colleagues (Yeh et al., 2008) have identified HCN1, HCN2 and HCN3 proteins in several types of ovary cells (oocytes, granulosa cells, theca cells, and corpora lutea, Table 1) with an expression pattern depending on the maturation state of the oocytes. HCN4 was instead identified only in oocytes and its expression level declined throughout the reproduction lifespan (Yeh et al., 2008). Interestingly, an indirect evidence supporting the functional role of HCN channels comes from the observation that the widely used chemotherapy drug cisplatin causes a dose-dependent decrease of HCN2 expression in antral follicle granulosa and thecal cells and of HCN4 in oocytes (Yeh et al., 2009). Although this effect is clearly not the only one associated with the ovarian damage induced by cisplatin, it strongly suggests the involvement of the HCN channels/I_f current in oocytes (patho) physiology, and provides an additional stimulus to identify the exact role of this current.

The presence of a HCN-like channel, named SpIH, has also been found in the tail of Sea urchin sperm (Gauss et al., 1998). Expression studies showed that the SpIH current shares many features with the mammalian I_f, but it also has the unique property of a rapid and incomplete inactivation. Curiously, the well-known direct HCN modulator cAMP removes the inactivation and increases the channel conductance without significantly affecting the voltage dependence of the activation. The authors speculate that the role of this channel might be to respond to the membrane hyperpolarization and cAMP increase induced by the interaction between the sperm and the Speract molecules released by sea urchin eggs, to control the flagellar beating and thus sperm movement towards the eggs (Gauss et al., 1998). It is however important to mention that so far, neither HCN nor HCN-like channels have been reported in human sperm.

3. HCN channels in left-right patterning during zygote development

It is well-known that bioelectrical signals provide, together with biochemical gradients, important cues in determining proper *in vivo* morphogenesis (Levin, 2014; Levin and Stevenson, 2012), and particularly the HCN4 currents represent one of these instructive signals for the *in vivo* left-right embryo patterning process. In *Xenopus laevis* embryos, HCN4 is expressed at a very early stage (2-cell stage) and its specific downregulation, through injection of a dominant negative (DN) HCN4 mRNA, into either one of the two blastomeres, resulted in cardiac mis-localization of the morphogenetic patterning genes *Xnr-1, Lefty, Pitx2, and BMP-4* (Pitcairn et al., 2017). In a different study, the same group extended the results beyond heart morphogenesis and demonstrated that HCN4 channels are widely expressed in *Xenopus* embryos up to embryonic stage 9. This evidence agrees well with the observation reported by Pai et al. (2017) that pharmacological inhibition of the f-current (with either ZD7288, 100 μM or ivabradine, 400 μM) at early embryo stages (stage 1−10) caused gut, heart, and gallbladder heterotaxia (*situs inversus*) in ~86% of tadpoles. Inhibition of HCN4 at more advanced stages (stages 10−40) instead did not cause any significant increase in heterotaxia compared to non-treated embryos (Pai et al., 2017).

Here, and in several other experiments described in this review, f-channel blockers (Bucchi et al., 2007) have been employed as a tool to identify the role of the f-current in the functional context of the cells/tissue under investigation. It is of capital importance to be aware that f-channel selectivity is valid within certain range of concentrations (Fig. 1) which may be profoundly influenced according to the type of experiment carried out (single cells or in multicellular/tissue).

4. HCN channels in cell proliferation and apoptosis: evidence from stem and cancer cells

Since its original discovery in the Sinus Node and in neurons, the role of the I_f current has been associated with the excitability of terminally differentiated cells. However, in more recent years, the presence of f/HCN channels has been also reported in Embryonic Stem Cells (ESC) that are unspecialized, pluripotent cells endowed with almost unlimited self-renewal capability but able to differentiate into the derivatives of all three germ layers, when exposed to appropriate stimuli. The recognition of these cells as a potentially unlimited source of new cardiomyocytes, both for basic research and for cell-based therapies, prompted the characterization of their electrical properties. The functional expression of f/HCN channels in undifferentiated ESC (Table 1) was addressed for the first time in 2005 by Wang et al. (2005). These authors reported that 30% of mouse mESCs exhibit a modest hyperpolarization-activated inward current, which was reversibly inhibited by the I_f blockers Caesium (Cs^+) and ZD7288. RT-PCR analysis carried out in mESC revealed the expression of the HCN2 and HCN3 isoforms, with a prevalence of HCN3; HCN1 and HCN4 signal were not detected **(**Table 1**)** (Wang et al., 2005). The kinetic features of the current, together with its lack of response to isoproterenol (1 μM) modulation, further supported a prevalent role of the cAMP insensitive HCN3 isoform. This conclusion was then confirmed by Lau et al. (2011) with protein expression studies. Different results were however obtained by Barbuti et al. (2009) who identified all four HCN transcripts in mESC but could not record any detectable current. Contradictory results were also found in relation to the HCN expression in human (h)ESC. While Wang and colleagues (Wang et al., 2005) failed to detect the presence of f/HCN transcripts and current in hESC, Sartiani et al. (2007) reported both the expression of HCN1, 2 and 4 channels

Table 1
Comprehensive overview of HCN channels expression and I_f current properties in the cellular and subcellular systems reported in this review. N.D. = not detectable; empty space = not assessed; *, by eye; §, recordings performed at external high potassium; dev = during development.

Subtype	HCN mRNA				HCN protein				amplitude-density	V half (mV)	Refs
	1	2	3	4	1	2	3	4			
Oocytes					x	x	x	x			(Yeh et al., 2008)
Rat											
Granulosa, and techal cells; corpora lutea					x	x	x				
Rat											
Embryonic stem cells	N.D/ x	x	x	N.D/ x		N.D	x		N.D/−2.2 ± 0.4 pA/pF @ −120 mV		(Lau et al., 2011; Wang et al., 2005; Barbuti et al., 2009)
Human	N.D/ x	N.D./ x	N.D.	N.D./ x	x	x		x	N.D./-3 pA/pF * @ −130 mV	−99.5 ± 1.4	(Wang et al., 2005; Sartiani et al., 2007)
Neuronal stem cells	N.D	x	x	N.D		x	x				(Johard et al., 2020)
Mouse											
Human		x	x								
Mitochondria					N.D.	N.D.	x	N.D.	−100 pA * @ −120 mV§		(León-Aparicio et al., 2019)
Human embryonic kidney (HEK)											
Rat renal cortex					N.D.	x	x	N.D.	−180 pA * @ −120 mV§		
Rat Cardiomyocytes					N.D.	x	x	x	−20 pA * @ −140 mV	−95 ± 7	Padilla-Flores et al., 2020
Pituitary Cells	x	x	x	x	N.D/ x	x	x	x			(Kretschmannova et al., 2012; Calejo et al., 2014)
Rat - mixed population											
Rat- lactotrophs									−1.5 ± 0.4 pA/pF @ −120 mV §	−94.1 ± 2.7	(Kretschmannova et al., 2012; Gonzalez-Iglesias et al., 2006)
Rat-gonadotrophs									−1.1 ± 0.1 pA/pF @ −120 mV §	−83.2 ± 3.3	Kretschmannova et al., 2012
Rat-somatotrophs									−1.7 ± 0.3 pA/pF @ −120 mV § −2.4 ± 0.4 pA @ −120 mV	−93.2 ± 3.4	(Kretschmannova et al., 2012; Simasko and Sankaranarayanan, 1997)
Rat-thyrotrophs									−2.8 ± 0.6 pA/pF @ −120 mV §		Kretschmannova et al., 2012
Pancreas	x	x	x	x							(El-Kholy et al., 2007)
Rat, mouse islets											
β-cells Mouse (MIN6)	x	x	x	x					−36.4 ± 6.1 pA/pF @ −130 mV§	−87.1 ± 1.7	
Rat β-cells					x	x	x	x	−37.4 ± 4.6 pA/pF @ −130 mV§ −50 pA * @ −140 mV	−84.3 ± 3.9	(El-Kholy et al., 2007; Zhang et al., 2009)
Human β-cells	x			x	x						Dorrell et al., 2016
Rat α- cells						x					Zhang et al., 2008
α- cells-Mouse (α-TC6)	x	x	x	x		x			−100/-300 * pA @ −140 mV§	−102 ± 1	
Taste Receptors	x	N.D.	N.D.	x	x			x	−70 pA* @ −140 mV	−105 ± 5	(Stevens et al., 2001)
Rat											
Astrocytes	x	x	x	x	x	N.D	N.D	N.D	−73.3 ± 29.6 pA @ −150 mV		(Honsa et al., 2014; Rusnakova et al., 2013)
Mouse Cortex											
Rat Cortex, Spinal cord									−900 pA * @ −140 mV	−98	Guatteo et al., 1996
Rat Hippocampal					N.D	N.D	N.D	x dev	−103.3 ± 32.7 pA @ −150 mV		(Honsa et al., 2014; Seo et al., 2015)
Microglia	x	x	x	x	x	x	x	N.D			(Vay et al., 2020)
Rat											
Osteoclast	x	N.D	N.D	x				x	−70 pA * @ −140 mV		Notomi et al., 2015
Mouse											
Uterine SMC									−6 pA/pF * @-130 mV	−84.3	(Okabe et al., 1999)
Rat circular											
Rat longitudinal									0/-1 pA/pF* @-120 mV		(Satoh, 1995; Okabe et al., 1999)
Corpus cavernous SMC							x	x			(Gur et al., 2019)
Human											
Lymphatic SMC	x	x	x	x	x	x	x	x			(Negrini et al., 2016)
Rat											
Sheep									−50 ± 12 pA @ −120 mV	−81 ± 1.8	McCloskey et al., 1999
Gastro-intestinal (ICCs)					N.D	x	x	x			(O'Donnell et al., 2015)
Human Colon											
Mouse Colon	N.D./ x	N.D./ x	x	N.D./ x				x			(Shahi et al., 2014; Lee et al., 2017)
Mouse Small intestine	N.D	N.D	N.D	N.D							Shahi et al., 2014
Mouse Jejunum	N.D.	x	x	x				x			Lee et al., 2017
Gastro-intestinal (Enteric neurons)					N.D	x	x	x			(O'Donnell et al., 2015)
Human Colon											
Mouse	x	x	x	x	x	x	N.D	N.D.			(Xiao et al., 2004; Yang et al., 2012)
Rat					x	x	N.D	N.D.			Xiao et al., 2004
Guinea pig					N.D.	x	N.D.	x	Myenteric: 305 ± 48.7 pA @ −100 mV	Myenteric: −85	(Galligan et al., 1990; Xiao et al., 2004)

(*continued on next page*)

Table 1 (*continued*)

Subtype	HCN mRNA				HCN protein				amplitude-density	V half (mV)	Refs
	1	2	3	4	1	2	3	4			
									Submucous: 172 ± 80 pA @-120 mV	Submucous: −95 ± 5.5	
Intestinal (SMC)									−50 pA *@ −120 mV	−91 *	Yanagida et al., 2000
Guinea pig -Circular											
Rabbit -Longitudinal									−100 pA * @ −100 mV	- 84	Benham et al., 1987
Kidney	x	x	x	x	x	x	x	x			(Calejo et al., 2014; López-González et al., 2020)
Rat whole-tissue											
Rat collecting duct cells	x	x	x	x		x			−500 pA * @ −140 mV	−102 ± 2	(Bolívar et al., 2008; Carrisoza-Gaytán et al., 2011; Uawithya et al., 2008)
Rat Proximal tubule					x		x				López-González et al., 2016
Rat thick ascending limb of Henle					N.D.		x				López-González et al., 2016
Ureteral putative pacemaker cells					x			x			He et al., 2018
Human											
Mouse							x				Hurtado et al., 2014
Bladder (ICCs)					x	x	x	x			Xue et al., 2012
Human											
Rat	x	x	x	x	x	x	x	x	−50 pA * @ −120 mV		(He et al. 2012, 2018; Dong et al., 2016; Deng et al., 2015; Lu et al., 2020)
Mouse					x			x			Wu et al., 2017
Bladder (Mucosae)	x	x	x	x	x	x	x	x			Kashyap et al., 2015
Human											
Rat	x	x	x	x	x	x	x	x			Kashyap et al., 2015
Bladder (detrusor SMC)	x	x	x	x	x	x	x	x			Kashyap et al., 2015
Human											
Rat	x	x	x	x	N.D./ x	N.D./ x	N.D./ x	N.D./ x	−200 pA * @ −140 mV	−74	(Green et al., 1996; Kashyap et al., 2015; He et al., 2012; Dong et al., 2016)
Mouse	x	x									Al-Naggar et al., 2019
Leukocytes	N.D.	x	x	N.D.							Fig. 3 this review
Human total Leukocytes											
Human Lymphocyte	N.D.	x	x	N.D.							
Human Granulocyte	N.D.	N.D.	x	N.D.							

and the corresponding I_f current in hESC (Table 1). Obviously, these studies paved the way to further investigations with the aim to unravel the role of this current in ESC. Studies in pluripotent mESCs, demonstrated the involvement of HCN3 channels in cell cycle progression; indeed, blocking the associated current with either ZD7288 (0.1–30 μM) or Cs^+ (1–10 mM) caused a dose-dependent decrease of cell proliferation (Lau et al., 2011). For example, 10 μM ZD7288, significantly increased their doubling time (from 28 to 37 h) by prolonging the S phase at the expenses of the G2/M phase (Fig. 2) (Omelyanenko et al., 2016). Prolongation of the S phase was caused by a slower rate of the DNA replication process, indicating a role of HCN channels in cell cycle progression, however the pharmacological block of I_f did not affect either the expression of pluripotency markers, or the capability of the mESC to differentiate into derivatives of the three germ layers (Lau et al., 2011; Omelyanenko et al., 2016).

Altogether the most relevant observations carried out in studies on cell cycle were: i) cells were more hyperpolarized during the S phase than during the G0/G1 phase (Lau et al., 2011; Ng et al., 2010) and ii) a sustained hyperpolarization of the membrane (corresponding to a block of the I_f current) induced mitotic arrest that could be reverted by membrane potential depolarization (Sundelacruz et al., 2009). Based on these experiments it is thus possible to speculate that membrane depolarization at the end of the S phase represents a necessary step for the transition to G2/M phase.

The presence of f/HCN channels has recently been reported also in mouse neuronal stem cells (mNSCs) (Table 1). In these cells HCN2 represents the dominant isoform and its expression increases during the progression of the cell cycle (Johard et al., 2020). Block of the HCN current halted the proliferation of mNSC and caused their accumulation in the G0/G1 phase (Fig. 2). In agreement with this observation, the arrest of cell cycle in G0 was characterized by alteration of markers of active proliferation (down-regulation)and of quiescence (up-regulation). Also, genes associated with differentiation processes were not upregulated, supporting the evidence that HCN block did not induce differentiation (Johard et al., 2020). It is worth noting that, despite these channels are significantly involved in cell cycle progression both in mESC and in mNSC, the isoforms involved are different.

Cell cycling is a central issue in cancer development and therefore the HCN contribution to this event may represent a novel potential pharmacological target. Johard et al. (2020) have recently shown that, in healthy NSC exposed to radiotherapy, the block of HCN channels (ZD7288, 10 μM) exerted a protective effect increasing their survival; however, when the same experimental protocol was applied to brain tumour cells (which also express HCN channels), radiotherapy fully maintained its ability to decrease cell survival. A clear understanding of the role of HCN channels in cancer cell proliferation is still lacking even if emerging evidence confirms their functional relevance. For example, Norberg and colleagues (Norberg et al., 2010) have shown that in a cellular model of a non-small-cell lung carcinoma (NSCLC), protein kinase C (PKC) inhibition, induced by the chemotherapy drugs staurosporine and PKC412, caused cell apoptosis due to a prolonged Ca^{2+} import mediated by HCN2 channels. Indeed, the effect of chemotherapy drugs was abolished when NSCLC cells were transfected with a siRNA specifically downregulating HCN2; similarly, transfection of HEK cells with HCN2 made these cells sensitive to staurosporine and PKC412. According to the authors, the drug-induced Ca^{2+} increase mediated by HCN2 channels represents the trigger for the mitochondrial release of apoptosis-inducing factor (AIF), followed by cell death. Although HCN channels mostly conduct Na^+ and K^+, HCN2 and HCN4 are also permeable to Ca^{2+} (Michels et al., 2008),

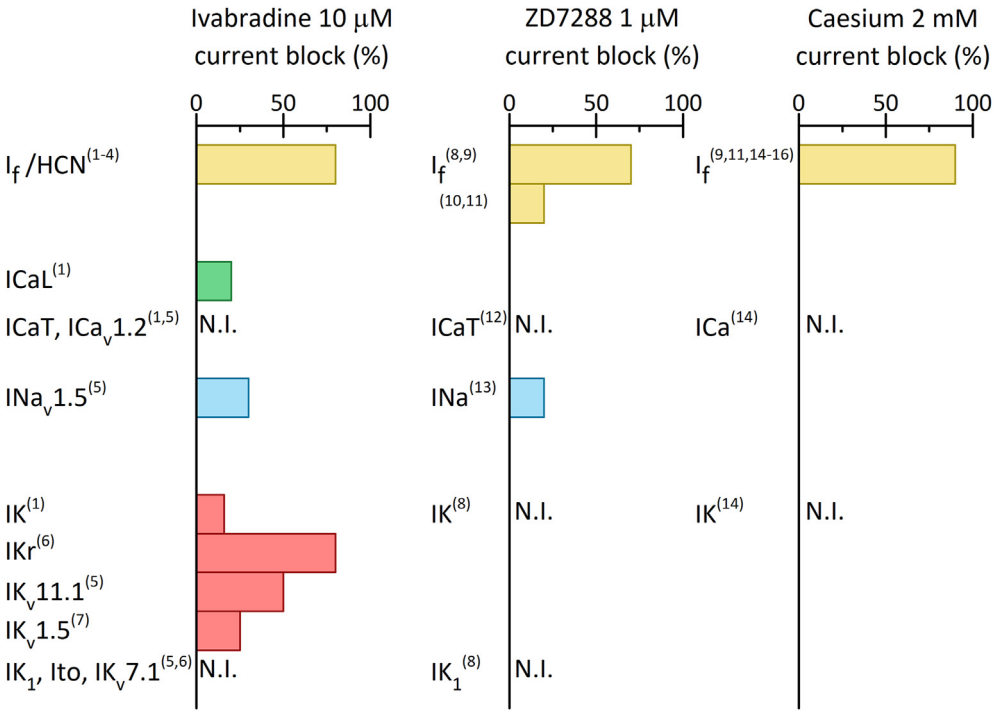

Fig. 1. Inhibitory effect of three "f-channels blockers" on the I_f and on a panel of additional currents. *Percentage current reduction induced in different types of cells by ivabradine (10 μM), ZD7288 (1 μM), and Cs^+ (2 mM). In some cases the % of block at the indicated doses was extrapolated by eye from the dose-response curve; for simplicity, when multiple data were available and quantitatively coherent we decided to show the "average" inhibition. N.I.:No inhibition: in this case the drug was tested but it did not elicit any effect. Data were collected from the literature as follows: 1,* (Bois et al., 1996)*; 2,* (Demontis et al., 2009)*; 3,* (Hsiao et al., 2019)*; 4,* (Stieber et al., 2006)*; 5,* (Haechl et al., 2019)*; 6,* (Koncz et al., 2011)*; 7,* (Delpón et al., 1996)*; 8,* (BoSmith et al., 1993)*; 9,* (Harris and Constanti, 1995)*; 10,* (Gasparini and DiFrancesco, 1997)*; 11,* (Satoh and Yamada, 2000)*; 12,* (Sánchez-Alonso et al., 2008)*; 13,* (Wu et al., 2012)*; 14,* (Denyer and Brown, 1990)*; 15,* (DiFrancesco, 1982)*; 16,* (Giannetti et al., 2021).

and Ca^{2+} permeability increases when HCN2 channels, that are normally phosphorylated by PKC at Threonine 549, become dephosphorylated (Norberg et al., 2010). This specific cell death mechanism, triggered by PKC inhibitors and mediated by HCN2, has also been found in primary cultures of rat cortical neurons (Norberg et al., 2010). Although unrelated to cancer, the Ca^{2+} influx through HCN channels followed by the AIF-mediated apoptosis has also been proposed as the mechanism behind the degeneration of Spiral Ganglion Neurons (SGNs) associated with the age-related hearing loss (Shen et al., 2018). Shen and colleagues demonstrated that HCN1 and HCN2 increased their expression in SGNs of old mice, and this increase correlated with the presence of AIF in the SNG nuclei. Further support to this mechanism comes from the observation that AIF-mediated degeneration of SNGs is increased by cAMP-induced activation of HCN channels by the adenylyl cyclase agonist forskolin (20 μM) (Shen et al., 2018). Lastly, it is worth mentioning that DNA-methylation analysis in colorectal cancer metastasis identified HCN4 as a biomarker for a poor prognosis (Ili et al., 2020).

5. HCN channels in cellular respiration and energy production

Mitochondria, the powerhouse of the cells, generate large amount of ATP through the cellular respiration process. In the matrix side of the inner mitochondria membrane (IMM), the presence of a negative potential ($\Delta\Psi m$, between −120 and −180 mV) is a necessary condition for the occurrence of oxidative phosphorylation since it provides a large driving force for mitochondria K^+ uptake which is necessary to counteract the flux generated by the K^+/H^+ antiporters. However, the evidence that under physiological conditions, most of the known mitochondrial K^+ channels are closed (Padilla-Flores et al., 2020) prompted to search for novel candidate for K^+ entry. Recently, two proteomic profiling studies of mitochondrial proteins identified several interacting partners of the HCN channels (León-Aparicio et al., 2019; Padilla-Flores et al., 2020); based on this evidence the authors proposed that HCN channels may provide a background K^+ influx into the IMM. The HCN isoforms expression in mitochondria depends on the specific cellular type: for example, HCN2, and HCN3 are expressed in the kidneys (León-Aparicio et al., 2019), while HCN2, HCN3, and HCN4 are present in cardiomyocytes, and HCN3 is the most abundant (Padilla-Flores et al., 2020) (Table 1).

Patch-clamp analysis carried out in both kidney mitochondria and cardiomyocyte mitoplasts, revealed a consistent ZD7288-sensitive hyperpolarization-activated current (Table 1). Interestingly, individual overexpression of single HCN isoforms in mitochondria of HEK293 cells shows that only HCN3 determines a current with kinetic properties similar to those recorded in the mitochondria of the renal cortex.

The expression level of HCN3 channels in kidney mitochondria increases during metabolic acidosis which is known to cause mitochondrial stress and selective autophagy driven by the depolarization of the IMM. Lopez-Gonzalez and collaborators (López-González et al., 2020) speculated that the increased expression of mitoHCN3 may be required to dissipate the membrane potential, thus initiating the mitochondrial autophagic process.

In cardiac mitoplast, instead, the kinetic properties of the I_f current were coherent with the functional expression of HCN2/HCN3/HCN4 hetero tetramers. Block of HCN channels by ZD7288 further hyperpolarized the $\Delta\Psi m$ and decreased the mitochondrial O_2 consumption (León-Aparicio et al., 2019; Padilla-Flores et al., 2020).

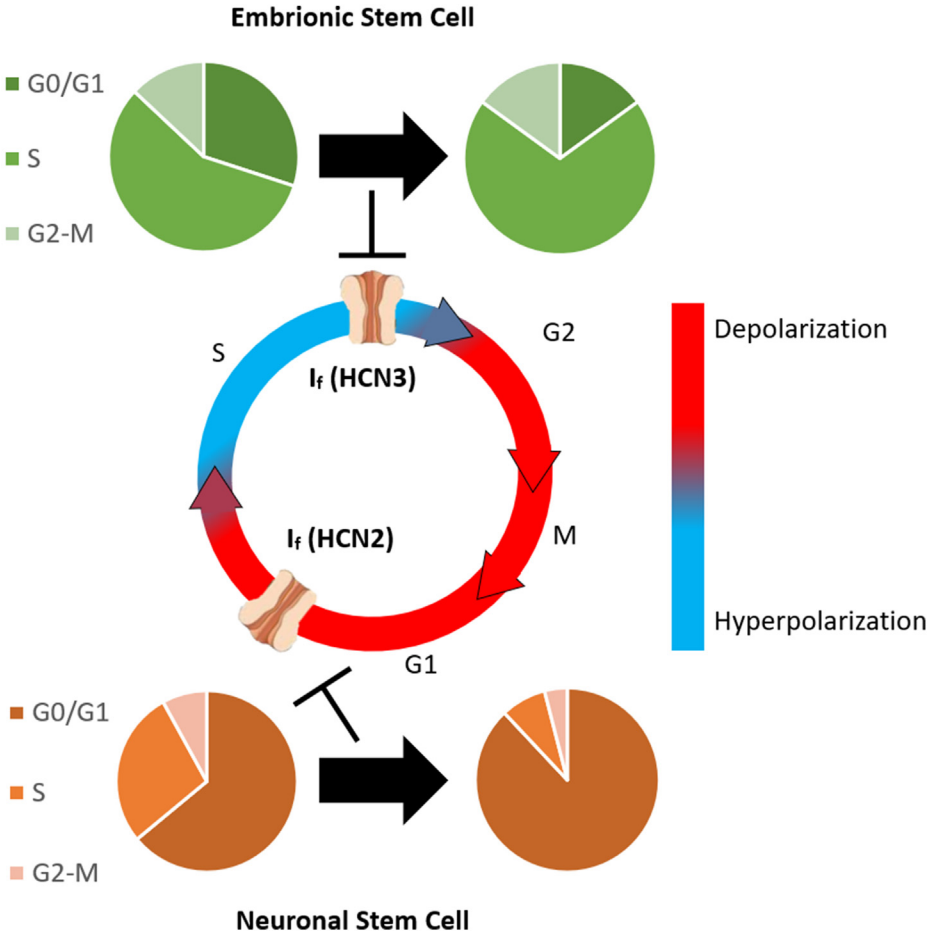

Fig. 2. Effect of *HCN channel block in the cell cycle progression of mouse Embryonic Stem Cells and Neuronal Stem Cells. Block of HCN3 and HCN2 channels expressed in mouse embryonic stem and neuronal stem cells, respectively, induces cell proliferation arrest with accumulation of cells in either the S phase (mESC) or in the G0/G1 phase (mNSC). Membrane voltage during normal cell cycle: blue corresponds to the hyperpolarized state and red to the depolarized state. See text for more details.*

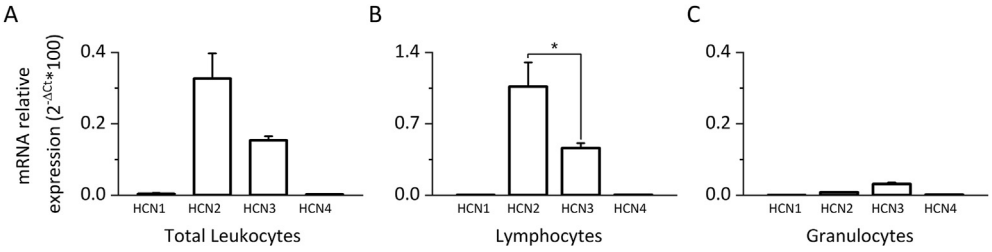

Fig. 3. HCNs mRNA profiling in human leukocytes. Relative HCN mRNA abundance assessed by means of qRT-PCR carried out in human total leukocytes (A) and in pure populations of human lymphocytes (B) and granulocytes (C) isolated from blood samples of 5 healthy subjects. Lymphocytes and granulocytes were isolated by flow cytometry. In all experiments, HCN mRNA expression was normalized to α-Tubuline (housekeeping gene) using the $2^{-\Delta Ct}$ method. Reproducible and reliable quantifications could be obtained only for HCN2 and HCN3 with the exception of HCN2 in granulocytes. *p < 0.05, Student-test.

In conclusion, according to these studies, HCN channels, and specifically HCN3, are part of the background conductances that maintain a proper K^+ influx into the IMM of mitochondria thus allowing the optimal $\Delta\Psi m$ for ATP synthesis favouring cellular respiration and ATP production.

6. HCN channels in endocrine cells

Secretory pathways are fundamental physiological processes allowing the transport of proteins and lipids from intracellular organelles to the plasma membrane where they can be released in the extracellular space. The membrane expression of HCN channels and their involvement in the regulation of exocytotic processes has been studied in different systems (Table 1); below an overview of these studies is provided.

6.1. Anterior pituitary cells

There are six types of endocrine anterior pituitary cells and they are named according to the primary hormones which are secreted:

gonadotrophs, corticotrophs, melanotrophs, thyrotrophs, somatotrophs, and lactotrophs. Each of these cell types has a typical action potential profile (tall and sharp single spikes or a variety of bursting activity) that are associated with different waves of intracellular Ca^{2+} oscillations ultimately resulting in specific pattern of hormones release (Fletcher et al., 2018). The mechanisms that control the complex electrical activity and the exocytosis of these cells are still not clear, however they require cell-type specific differential expression of ion channels. Several currents have been proposed as modulators of the spontaneous firing, and the I_f current may be one of them (Fletcher et al., 2018). All four HCN transcripts were detected in a heterogeneous population of rat anterior pituitary cells cultured for 24 h, and HCN2, HCN3 and HCN4 proteins were identified by Western blot (Kretschmannova et al., 2012) (Table 1). Patch-clamp studies recorded the I_f current in the majority of cultured thyrotrophs, gonadotrophs, and somatotrophs cells (Kretschmannova et al., 2012; Simasko and Sankaranarayanan, 1997), while experiments performed in freshly isolated and cultured rat lactotrophs cells indicated that I_f was present only in a fraction of cells (30–40%) (Gonzalez-Iglesias et al., 2006; Kretschmannova et al., 2012; Simasko and Sankaranarayanan, 1997) (Table 1). The effect of the I_f block on the spontaneous activity of pituitary cells was evaluated in several studies, but conflicting results were obtained and only some of them reported a reduction in the firing frequency (Chu et al., 2010; Kretschmannova et al., 2012; Simasko and Sankaranarayanan, 1997). This discrepancy clearly points out that further work is needed to improve our understanding of the I_f function in the spontaneously activity pituitary cells.

Another important functional pathway which may involve the contribution of the I_f current consists in the molecular events that couple the activation of G-protein coupled receptors (GPCR) to the secretory activity of pituitary cells (Kretschmannova et al., 2012). Indeed, agonists of GPCRs are physiological regulators of the electrical activity of these cells and it is known that G_s or G_i-dependent changes in cAMP and Gq/11-dependent depletion of PIP2 can regulate HCN channels (Baruscotti et al., 2005; Pian et al., 2007). Interestingly, Calejo and collaborators (Calejo et al., 2014) reported the expression of HCN2 channels both in the plasma membrane and in (or nearby) the membrane of secretory vesicles of rat lactotrophs cells. Functional studies aimed at investigating a possible involvement of HCN current in exocytosis were performed by increasing the HCN current density in rat lactotrophs cells (via HCN2 over-expression) and by inhibiting the current with ZD7288 (100 μM). These studies concluded that the exocytic activity is likely regulated by localized increases of Ca^{2+} levels caused by permeation through HCN2 channels.

6.2. Pancreatic cells

Pancreatic islet cells have a dynamic electrical activity which is pivotal for hormones secretion in response to metabolic changes; HCN channels have been found among the ion channels expressed on β- and α-cells membranes that may thus contribute to insulin and glucagon secretion (Dorrell et al., 2016; El-Kholy et al., 2007; Zhang et al., 2008, 2009) (Table 1). All HCN isoforms have been detected at the mRNA level in mouse and rat islets with HCN2 being the predominant subunit in the mouse, and HCN3 and HCN4 the main isoforms in the rat. Immunostaining experiments carried out in the rat model, showed that β-cells express all HCN isoforms; investigation in α-cells was limited to HCN2 and its presence was confirmed. In humans, the scenario is somewhat different since only HCN1 and HCN4 are present and differentially express depending on the β-cell subtypes (Dorrell et al., 2016). In rat β-cells and in clonal TC6 α-cells, a HCN-mediated inward current has been characterized and the canonical I_f properties have been reported (Table 1) (El-Kholy et al., 2007; Zhang et al., 2008). Even though the specific role played by the current in these cells is still unclear, a possible mechanism may involve the regulation of the membrane input resistance (Zhang et al., 2009). Dorrell and colleagues (Dorrell et al., 2016) reported that HCN current inhibition (with 30 mM ivabradine) had a dual effect on insulin secretion in human islets: during basal condition, ivabradine increased insulin secretion, while a reduction was observed during glucose-stimulation. However, when the effect of the HCN block was tested in isolated β-like cells generated from human pluripotent precursors, only the stimulation of basal secretion activity was observed (Dorrell et al., 2016). Data collected in rat islets showed somewhat different results; indeed HCN2 over-expression increased basal insulin secretion, while suppression of HCN current, through overexpression of the dominant negative HCN2-AYA channels, decreased glucose-stimulated insulin secretion only at low (2.5 mM) external K^+ concentrations (Zhang et al., 2009). Comparison between data obtained in human and rat islets indicate that either HCN channels may have species-specific effects or they may reflect different experimental conditions (e.g. over-expression, unspecific effects due to high ivabradine concentrations).

In mouse clonal TC6 α-cells, inhibition of HCN current with ZD7288 (100 μM) increased glucagon secretion, while stimulation of the HCN current by lamotrigine reduced glucagon release (Zhang et al., 2008). Similarly, HCN current inhibition by ZD7288 (100 μM) or cilobradine (5 μM) increased glucagon release from rat islets (Zhang et al., 2008).

Further investigations are required to properly identify the mechanisms linking HCN channels and exocytosis; however, possible roles may involve regulation of membrane input resistance, mixed Ca^{2+} and Na^+ influx, and/or cytoskeleton interaction between HCN channels and exocytotic machinery (Zhang et al, 2008, 2009).

7. HCN channels role in sour taste transduction

In vertebrates, the sour stimulus (i.e. protons) is detected by taste receptors by means of a transduction pathway that includes protein receptors and ion channels. The presence of the I_f current was first identified in rat in 2001 by Steven and collaborators (Stevens et al., 2001) who detected the expression of HCN1 and HCN4 proteins in a subset of taste receptors cells of the vallate papilla (Table 1). Three main elements indicate that HCN channels may play a role in sour taste transduction: *i*) the I_f current in these cells is increased by external sour stimuli, *ii*) lowering the extracellular pH induces a dose-dependent modulation of the activation curve of heterologously expressed HCN1 and HCN4 channels, *iii*) cells expressing HCN channels do not express the transduction elements involved in sweet and bitter taste detection (Stevens et al., 2001).

8. HCN channels in glial cells

The expression of HCN channels in neuronal cells of the Central Nervous System (CNS) has been extensively documented in a large number of studies, and several reviews provide detailed descriptions of their role (Biel et al., 2009; Santoro and Shah, 2020). Interestingly, the presence of these channels has also been reported in astrocytes and microglia.

Astrocytes are the prevalent glial cells in the brain, where they perform several regulatory functions (Kimelberg and Nedergaard, 2010). The first recording of an I_f-like current in cultured cortical and spinal cord astrocytes was accomplished in 1996 by Guatteo et al. (Table 1) (Guatteo et al., 1996), but only 17 years later

Rusnakova and colleagues (Rusnakova et al., 2013) detected the HCN mRNA in postnatal (P10 to P50) astrocytes. In 2015, Seo et al. (2015) analyzed the HCN isoform expression during hippocampal development and demonstrated the presence of HCN4-positive astrocytes in the *stratum lacunosum moleculare*. The evidence that HCN4 expression was lost at P21 was strongly suggestive of a stage-specific role in early astrogliogenesis. Interestingly, despite the fact that under physiological conditions adult glial cells express low or negligible I_f current, after focal cerebral ischemia, mRNA (HCN1, HCN2 and HCN3) and protein (HCN1 and HCN3) (Table 1) expression in astrocytes increased significantly both in rat hippocampus and mouse cortex (Honsa et al., 2014; Rusnakova et al., 2013). In isolated cortical astrocytes, 5 weeks after ischemia these changes in expression were accompanied by a depolarization of the resting potential, a decrease of membrane resistance (Rm), and the presence of a substantial ZD7288-sensitive I_f current. Similar effects were observed in hippocampal reactive astrocytes after global cerebral ischemia, even though in these cells the membrane resistance was not affected (Honsa et al., 2014). An increase of HCN1 and HCN2 immunoreactivity in GFAP + astrocytes was also recently reported in the hippocampus of gerbils 4 days after the induction of 5 min transient global cerebral ischemia (Park et al., 2019).

Microglia represents the tissue-resident macrophages in the CNS and mediate cell homeostasis and inflammatory processes. Microglia cells display a constant movement of their processes in order to detect insults and, based on different types of stimuli, they may induce either neurotoxicity or neuroprotection. Many ion channels and cell surface receptors shape these functions (Izquierdo et al., 2019). In a recent paper Vay and colleagues (Vay et al., 2020) provided the first evidence that isolated microglial cells express all HCN isoforms (Table 1), however, only HCN2 exhibited expression changes after pro- and anti-inflammatory stimuli. Curiously, HCN inhibition by ZD7288 (10–30 μM) resulted in an unexpected depolarization of the cell membrane potential and a sustained increase of intracellular Ca^{2+}. This evidence led to speculate that, as observed in some neurons, a sustained background HCN current may both reduce Rm and depolarize the cell thus influencing the gating state of other voltage-dependent channels (e.g K^+ and Ca^{2+} channels). The membrane hyperpolarization expected upon HCN blockade could therefore be masked by activation/inactivation of these channels, whose effect on membrane voltage may be amplified by the increased Rm caused by the block of HCN channels. In this study, the authors also showed that differential expression of HCN isoforms is differently regulated by pro- and anti-inflammatory polarization of microglia (Vay et al., 2020). HCN2 channels were significantly downregulated by a pro-inflammatory stimulus and strongly upregulated by an anti-inflammatory stimulus, HCN3 expression was reduced in both conditions, and HCN1 and HCN4 expression was not affected.

HCN block induced either by ZD7288 (10–30 μM) or by siRNA-mediated HCN2 silencing decreased the expression of the pro- and anti-inflammatory markers stimulated by treatments with lipopolysaccharides (LPS) and interleukin-4 (IL4), respectively. These data suggest that inhibition of HCN, primarily HCN2, hinders the correct activation of microglia, likely by a modification of intracellular Ca^{2+} signalling. The switch of microglial cells from a resting to an active state is also characterized by cell proliferation, however this process was limited by the inhibition of HCN channels by 30 μM ZD7288 (Vay et al., 2020).

9. HCN channels in osteoclasts

Bone resorption requires osteoclasts differentiation (osteoclastogenesis) and activation (Boyle et al., 2003). An interesting observation made by Notomi et al. (2015) in mouse osteoclast precursor-like cells (RAW 246.7 monocyte macrophage lineage osteoclast precursor cells) revealed that, in the presence of RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand), membrane hyperpolarization accelerates their differentiation toward osteoclasts. The same group also reported the expression of HCN4 proteins both in RAW cells and in mouse bone marrow–derived osteoclasts; furthermore, the HCN-mediated current was recorded in osteoclasts derived from RAW 246.7 cells (RAW-OC) (Table 1).

Functional studies indicated that HCN channels activation inhibits the hyperpolarization-induced osteoclastogenesis process (Notomi et al., 2015). An additional role of HCN channels in the modulation of osteoclastogenesis was revealed analysing the prostaglandin E2 (PGE2) pathway. While PGE2 stimulates the differentiation of osteoclasts (Kobayashi et al., 2005), it also activates the production of cAMP, which increases the open probability of HCN channels. Interestingly, when the contribution of HCN to membrane potential of RAW cells is removed by siRNA-mediated HCN4 knockdown, PGE2-induced osteoclastogenesis is potentiated.

10. HCN channels in smooth muscles

10.1. Uterus

Myometrial smooth muscle displays an intrinsic pacemaker activity which drives uterine contractions, and several factors, including hormones, stretch, and paracrine molecules can modulate this activity. Uterine contractions occur both in the non-pregnant state (during the menstrual cycle) and during gestation, and according to different physiological or pathophysiological conditions they vary in frequency, duration, amplitude, and direction of propagation (Aguilar and Mitchell, 2010; Wray and Prendergast, 2019). To date, the mechanism behind the pacemaker activity of the uterus is still largely unknown, and neither an anatomical pacemaker region, nor the presence of widely distributed pacemaker cells, have been identified. However, based on the evidence that small isolated myometrial strips show spontaneous rhythmic contractions, it is likely that some myocytes are endowed with an intrinsic pacemaker activity (Wray and Prendergast, 2019; Young, 2018).

The involvement of the I_f current in myometrial automaticity was suggested in the 1990s when the current was measured in freshly isolated single longitudinal and circular smooth muscle cells isolated from pregnant rat uterus (Table 1) (Okabe et al., 1999; Satoh, 1995). The I_f density was larger in circular than in longitudinal myometrial cells and this finding well correlates with the evidence that spontaneous APs were frequently observed in circular but not in longitudinal myocytes. This observation led to the hypothesis that the slow activation of the I_f current at resting membrane potential might cause a slow depolarization, driving the membrane potential to the threshold for AP firing. In agreement with this conjecture, Okabe et al. (1999) observed a reduction in the spontaneous contraction rate of circular muscles following Cs-induced I_f block (IC_{50} of 0.15 mM). Additional data indicated that I_f inhibition by ZD7288 (100–200 μM) decreased the force amplitude of spontaneous and oxytocin-induced contractions of longitudinal uterine strips isolated from in-term pregnant rats (Alotaibi et al., 2017). Unexpectedly, in addition to decrease the force amplitude, ZD7288 also increased the frequency of contractions. The interpretation of these results is not straightforward since ZD7288 was used at high concentrations and therefore non-specific effect on other ion channels are likely contributing.

10.2. Corpus cavernous

The beneficial effects of ivabradine on erectile dysfunction (ED) observed in patients with heart failure and in ApoE knockout mice,

have been attributed to the drug-induced reduction of heart rate, endothelial oxidative stress, and penile fibrosis (Baumhäkel et al., 2010; Mert et al., 2018). Recently, Gur and colleagues (Gur et al., 2019) verified the hypothesis that HCN channels may also be functionally expressed in the smooth muscle of the corpus cavernous. Their study indeed identified the presence of HCN3 and HCN4 proteins in smooth muscle cells of the human penile corpus cavernous (Table 1) and showed that ivabradine (100 nM-1 mM) was able to induce a concentration-dependent relaxation of tissue strips of the human corpus cavernous previously contracted by phenylephrine. This effect was independent from the Nitric Oxide (NO)/soluble guanylyl cyclase pathway since it was maintained in the presence of both nonspecific nitric oxide synthase (NAME) and soluble guanylyl cyclase (ODQ) inhibitors. Gur and co-workers thus proposed that the direct inhibition of HCN channels in penile tissue may contribute to the beneficial effects of ivabradine on ED, and that HCN block may be a useful treatment for patients who have cardiovascular risk factors and ED (Gur et al., 2019). In addition, they also reported the evidence that ivabradine may affect other ion channels such as L-type Ca^{2+} and K^{+} channels.

10.3. Lymphatic vessels

Smooth muscle cells of the lymphatic ducts walls exhibit spontaneous contractions that are preceded by either a single or a complex pattern of APs (Kirkpatrick and McHale, 1977; Ward et al., 1991). A similar activity was more recently confirmed by Telinius and colleagues (Telinius et al., 2015) in isolated thoracic and mesenteric human lymphatic vessels. The main feature of these APs is the presence of a slow depolarization process that drives the cell membrane potential from the most negative value, reached at the end of the repolarization process, to the threshold for the next AP. The first suggestion that a hyperpolarization-activated inward current similar to I_f might be involved in these spontaneous electrical oscillations came from a study of Allen and McHale, 1988 on bovine lymphatic ducts. However, it was only in 1999 that McCloskey (McCloskey et al., 1999) fully described the I_f current in freshly isolated sheep mesenteric lymphatic smooth muscle cells (Table 1), and showed that blockade of this current by Cs^{+} (1 mM) or by ZD7288 (1 μM) decreased the frequency of spontaneous contractions in intact lymphatic vessels. Similar results were obtained in rat smooth muscle cells of peripheral diaphragmatic lymphatic vessels by Negrini and co-workers (Negrini et al., 2016) who demonstrated the expression of all HCN isoforms (Table 1) and the ability of HCN channels blockers (Cs^{+}, ivabradine, and ZD7288) to reversibly decrease the rate of contraction and to increase the end-diastolic diameter in a dose-dependent manner. The authors proposed that, during the diastolic phase, the inward current carried by HCN channels contributes to depolarize the cell up to the threshold for Ca^{2+} channels activation, which would in turn trigger the lymphatic muscle contraction (Negrini et al., 2016). However, the functional role of HCN channels is still not clear as proved by data indicating that intermediate concentrations of ZD7288 increased, rather than decreased, the frequency of contractions, a phenomenon observed also in other types of smooth muscles, such as the bladder detrusor (Green et al., 1996) and the portal vein smooth muscle (Greenwood and Prestwich, 2002).

11. HCN channels in the gastrointestinal system

The presence of HCN channels has been widely reported in all the cells associated with gastrointestinal (GI) mobility and in particular in the interstitial cells of Cajal (ICCs), in enteric neurons, and in smooth muscle cells (Table 1).

11.1. Interstitial cells of Cajal

Interstitial cells of Cajal (ICCs) are specialized mesenchymal pacemaker cells organized in networks that are electrically coupled to smooth muscle cells and are distributed in several parts of the gastrointestinal wall. ICCs are able to generate a basic slow electrical rhythm that modulate the phasic contractions of gastrointestinal smooth muscle cells. According to Sanders et al. (2014), the pacemaker mechanisms responsible for the automaticity of ICCs involve both the spontaneous release of Ca^{2+} from intracellular stores and the activity of membrane ion channels such as Ca^{2+}-activated Cl^{-} channels (Ano1) and T-type Ca^{2+} channels. However, in 2014 the presence of HCN1 and HCN3 transcripts was successfully reported in cultured murine c-kit and Ano1 positive colonic ICCs cells by Shahi and co-workers (Shahi et al., 2014), and in 2015 HCN2, HCN3, and HCN4 proteins were detected by immunofluorescence staining in human colonic ICCs (O'Donnell et al., 2015). When mouse colonic ICCs were maintained in culture for 2–3 days, they exhibited spontaneous APs that were reduced by I_f inhibitors (5 mM Cs^{+}, 10 μM ZD7288, 10 μM zatebradine and 10 μM genistein). Taken together these data raised the hypothesis that HCN channels may participate in the generation of the colonic ICCs pacemaker activity. Interestingly, the same authors demonstrated that the I_f current inhibitors had no effect on spontaneous activity of small intestinal ICCs and this is in agreement with the lack of HCN channels expression in cultured c-kit and Ano1-positive ICCs of the small intestine. These results indicate that different pacemaker mechanisms likely contribute to the activity of different areas of the GI tract (Shahi et al., 2014).

Transcriptome analysis of freshly isolated cells from the murine *tunica muscolaris* of the jejunum and colon (JICCs and CICCs, respectively) reported positive signals for HCN2, HCN3, and HCN4 in both cell types (Table 1). HCN4 was the most abundant isoform in JICCs and the least expressed in CICCs; the presence of HCN4 was also confirmed at the protein level (Lee et al., 2017). In addition to the full HCN4 transcript, JICCs also express truncated HCN4 variants (HCN4v2 and HCN4v3) both encoding for the same 795 aa-long truncated polypeptide lacking the entire N-terminal and the S1–S4 segments.

HCN channels have also been proposed to regulate the pacemaker activity of ICCs of murine gastric antrum cells (Si et al., 2012), and the association between gastrointestinal pathologies and impaired expression of HCN channels in ICCs, strengthens the role of these channels in regulating gastrointestinal motility (Guo et al., 2017; O'Donnell et al., 2015). Guo and co-workers (Guo et al., 2017) indeed showed that mice with malignant ascites developed gastrointestinal dysmotility associated with impaired peristaltic activity likely caused by an alteration of the ICCs morphology and by a decreased expression of HCN2 and Ca^{2+} transient in these cells.

11.2. Enteric neurons

The presence of the I_f current was first described in guinea pig myenteric AH neurons and in a small fraction of both S and AH neurons of the submucous plexus (Galligan et al., 1990; Messenger et al., 1994). Since then, the presence of HCN channels in murine, guinea-pig, zebrafish, and human neurons of the enteric nervous system (ENS) has been reported in several studies (Table 1) (Fujii et al., 2020; O'Donnell et al., 2015; Xiao et al., 2004; Yang et al., 2012). Xiao et al. demonstrated that the HCN1, HCN2, and HCN4 isoforms are differently expressed in different neurons of the myenteric and of the submucosal plexuses, and their relative abundance depends on the species investigated (Xiao et al., 2004). A more detailed characterization of the HCN2 positive neurons in the GI tract of mice was performed by Yang and co-workers (Yang

et al., 2012) who reported the protein expression mainly in cholinergic neurons of the myenteric plexus that possess processes positioned in close proximity to ICCs. Although these authors did not present functional data, they suggested that HCN2 channels activation would facilitate the release of acetylcholine from cholinergic neurons and activate the muscarinic M receptors of ICCs thus affecting the peristalsis. O'Donnel and co-workers (O'Donnell et al., 2015) carried out immunofluorescence staining experiments in the human colon and identified the presence of HCN2, HCN3 and HCN4 channels in both the myenteric and submucosal plexuses. Interestingly, the same authors analyzed colon samples of patients suffering from Hirschsprung's disease and Western blot experiments showed a decrease of the HCN3 protein signal, indicating a possible involvement of this isoform in the pathophysiology of this disease. Experiments performed in the zebrafish model also suggested the involvement of the HCN4 channel of the enteric neurons in regulating retrograde peristalsis (Fujii et al., 2020).

11.3. Gastrointestinal smooth muscle cells

The I_f current was identified in isolated longitudinal smooth muscle cells of rabbit Jejunum (Benham et al., 1987) and in circular smooth muscle cells of guinea pig ileal muscle (Yanagida et al., 2000) (Table 1). Obviously, the presence of the current suggests a possible involvement in the slow depolarization observed in response to hyperpolarizing current stimuli, possibly caused by various inhibitory neurotransmitters and hormones.

12. HCN channels in the urinary system

HCN channels have been identified in various parts of the urinary system (Table 1): kidneys (Bolívar et al., 2008; Calejo et al., 2014a; Carrisoza-Gaytán et al., 2011; López-González et al, 2016, 2020), uretere (Hashitani et al., 2017; He et al., 2018; Hurtado et al, 2010, 2014), and bladder (Al-Naggar et al., 2019; Deng et al., 2015; Dong et al., 2016; He et al., 2012; Kashyap et al, 2015, 2020; Lu et al., 2020; Mader et al., 2018; Wu et al., 2017; Xue et al., 2012).

12.1. Kidneys

In 2008 Bolivar et al. (Bolívar et al., 2008) carried out the first recording of a hyperpolarization-activated voltage- and time-dependent inward current in primary cultures of rat inner medullary collecting duct (IMCD) cells. This current, dubbed I_{vti}, displayed the typical features of the I_f current: mixed K^+ and Na^+ permeability, voltage dependence of kinetics parameters, and modulation by cAMP and by $[K^+]_o$, however, it exhibited an anomalous pharmacological profile since it was insensitive to Cs^+ and ZD7288 block, but blocked by Cadmium (Bolívar et al., 2008). Attempts to identify the molecular nature of the current resulted in the identification of HCN1, HCN2 and HCN4 mRNA expression, however the presence at the protein level was investigated and confirmed only for HCN2 (Table 1). Whether the peculiar pharmacological profile of this channel is due to an alternative splicing form of HCN mRNA or to post-translational modification is at present unknown and extremely puzzling. Several other groups confirmed the expression of HCN2 and also of HCN3 channels in rat kidneys, and further studies described these channels as important players in the regulation of the acid–base and K^+ balance (Calejo et al., 2014a; Carrisoza-Gaytán et al., 2011; López-González et al, 2016, 2020; Padilla-Flores et al., 2020; Uawithya et al., 2008). The HCN signals were immunodetected on the basolateral membrane of acid-secreting intercalated and principal cells of the connecting tubule and of the collecting duct both in the cortex and in the medulla. When heterologously expressed in oocytes, HCN2 channels displayed permeability to NH_4^+ with a strong dependence on the extracellular NH_4Cl concentration and were inhibited by ZD7288 (Carrisoza-Gaytán et al., 2011). Despite the authors identified the HCN2 expression both in the acid-secreting intercalated and in principal cells, a specific role of HCN2 channels in regulating NH_4^+ excretion was confirmed only in the former cell type, where ZD7288 (10 μM) reduced the slope of acidification of the tubular contents. Based on this evidence, the authors suggested that the selective expression of HCN2 channels in the basolateral membrane of acid-secreting intercalated cells may represent a physiological mechanism favouring NH_4^+ uptake in the collecting duct and thus its urinary excretion (Carrisoza-Gaytán et al., 2011). Concerning the role of HCN channels in principal cells, the authors speculate that the entrance of Na^+ through HCN2 and HCN3 channels could provide a stimulus for promoting K^+ uptake via the basolateral Na^+-K^+-ATPase and its excretion through voltage-gated K^+ channels on the apical membrane (Carrisoza-Gaytán et al., 2011; López-González et al., 2020).

The expression of full length and N-terminal truncated HCN1 and HCN3 proteins has been reported in the apical membranes of cortical and medullary proximal tubules of rat nephrons (Table 1), and HCN3 was the sole isoform immunodetected in the medullary Thick Ascending Limb of Henle (MTALH) (López-González et al., 2016). Interestingly, the expression of HCN3 channel forms has been reported to be regulated by K^+ diet (López-González et al., 2016). Since a thorough discussion of the physiological roles of HCN channels in the various regions of the nephron is beyond the aim of the present work, we refer the interested readers to the original studies (Carrisoza-Gaytán et al., 2011; López-González et al, 2016, 2020). To summarize the main points of these studies, we can conclude that there is now solid evidence indicating that in mammalian kidney, HCN channels, and particularly HCN2 and HCN3, play an important role in regulating Na^+, K^+ and NH_4^+ homeostasis.

12.2. Ureteral tract

The progression of urine from the renal pelvis to the bladder depends on the coordinated peristalsis originating in the pelvis-kidney junction (PKJ (Hurtado et al., 2014, 2010),). A rhythmic activity is indeed initiated in the PKJ by specialized pacemaker cells that are coupled via gap junction to the surrounding smooth muscle cells. In a series of studies aimed at understanding the mechanism underlying the rhythmic activity, Hurtado et al. (Hurtado et al, 2010, 2014) reported a high level of expression of HCN3 proteins in PKJ cells. Despite no direct recording of the HCN3 current was carried out, the researchers used the optical mapping technique to demonstrate that 30 μM of ZD7288 caused a severe desynchronization of the proximal-to distal contraction and at a higher dose (90 μM) it completely blocked both the electrical activity and contractility (Hurtado et al., 2010). Similar results were obtained in studies on human ureteral preparations (He et al., 2018) where treatment with ZD7288 (50 μM) inhibited the amplitude but not the frequency of spontaneous contractions of ureteral smooth muscle strips. According to the authors the spontaneous contracting activity likely originates in putative pacemaker cells, the so called interstitial-like cells of Cajal (ICCs), also identified in the bladder (see paragraph 12.3 below), which express both HCN1 and HCN4 channels together with c-kit, a specific marker of ICC. However, as recognized by the authors, due to the lack of solid electrophysiological evidence, no definite conclusion on f-current function in these cells can be drawn (He et al., 2018).

12.3. Bladder

In 1996 an I_f–like, Cs^+ and ZD7288 sensitive inward-rectifying current with mixed Na^+/K^+ permeability was described in rat detrusor smooth muscle cells (Table 1) (Green et al., 1996). Since this initial observation several additional studies addressed the involvement of the HCN channels in the regulation of bladder excitability and reported the presence of all HCN isoforms in the detrusor muscle, ICCs, and in the mucosal layer of the urinary bladder (Al-Naggar et al., 2019; Deng et al., 2015; Dong et al., 2016; Kashyap et al, 2015, 2020; Lu et al., 2020; Mader et al., 2018; Wu et al., 2017; He et al., 2012; Xue et al., 2012) (Table 1). While the expression of HCN channels in the various regions of the bladder is therefore an established evidence, a thorough understanding of their role is still missing. In their original work, Green and collaborators (Green et al., 1996) revealed that a sustained treatment with the I_f blocker ZD7288 induced a dose-dependent increase of both the amplitude and, surprisingly, the frequency of spontaneous phasic contractions of rat detrusor muscle strips. More recently, Kashyap and co-workers (Kashyap et al., 2015) demonstrated that application of Lamotrigine (10 nM-100 μM), an anticonvulsant known to increase the HCN-mediated current, displayed a relaxant effect on the detrusor muscle of the rat bladder and that this effect was blocked by application of ZD7288 (10 μM). The I_f blocker caused a dose dependent increase in force of contraction, which was more pronounced in mucosa-free bladder strips, than in intact bladder strips. Similar studies carried out on human bladder strips confirmed the observations from the animal models (Mader et al., 2018). Indeed, this group demonstrated that exposure to ZD7288 (50 μM) caused tonic contractions and increased both the amplitude and the spontaneous frequency of phasic contractions, and that the activation of HCN channels with Lamotrigine induced relaxation in both mouse and human bladder, reducing the effect of ZD7288.

Recently, Kashyap et al. (2020) provided an integrated explanation for the role of HCN channels in the bladder and proposed that the HCN channels found in the mucosa, in the cholinergic nerve terminals, and in the detrusor muscle are constitutively active. According to these authors, the HCN current exerts an inhibitory effect both on neurotransmitters release and on spontaneous contractions of detrusor smooth muscle and these effects are likely due to an HCN-mediated attenuation of N- and T-type voltage-gated Ca^{2+} channels activity (Kashyap et al., 2020).

In contrast with these data, other authors reported that application of both ZD7288 (10–50 μM) and ivabradine (30–90 μM) induced relaxation of rat bladder strips (Aydin et al., 2018; Deng et al., 2015). Interestingly, Deng and colleagues (Deng et al., 2015) showed that in a pathological model of detrusor muscle overactivity, all HCN isoforms were upregulated and higher doses of ZD7288 was necessary to cause relaxation.

HCN channels distribution and function have been also investigated in the bladder of a rat model of diabetic cystopathy (DCP), a disease characterized by increased urinary frequency and incontinence (Dong et al., 2016); in this study a decreased HCN/I_f expression was observed in ICCs. In agreement with this finding, DCP ICCs were less sensitive to cAMP modulation induced by forskolin (a permeable Adenylyl Cyclase agonist) and to ZD7288 treatment. Of note, this decrease in HCN expression has been associated with a DCP-dependent decrease in the number of caveolae (and of caveolin-3 levels) and knockdown of caveolin-3 in control ICCs elicited a phenotype similar to that of DCP since it decreased both the HCN protein expression and the I_f current (Dong et al., 2016). This finding is in agreement with previous works demonstrating that caveolin-3 interacts with and modulates HCN function, and impairment of such interaction decreases the expression of channels (Barbuti et al, 2007, 2012).

13. HCN channels in leukocytes

Several studies have elegantly shown that the I_f blocker ivabradine, a drug used in the clinic to reduce heart rate, also displays relevant effects on endothelial functions, oxidative stress, and lymphocytes migration (Bonadei et al., 2018; Dallapellegrina et al., 2020; Li et al., 2016; Walcher et al., 2010). For example, ivabradine treatment of transgenic ApoE-deficient mice resulted in a protective action against atherosclerosis progression by activating anti-inflammatory pathways (Aquila et al., 2018; Custodis et al., 2008). In another study carried out in a mouse model of cardiac inflammation, the authors reported that ivabradine prevented the progression from myocarditis to dilated cardiomyopathy, and this effect was associated with a reduction of the p38 MAPK pathways and a downregulation of several pro-inflammatory cytokines (Dallapellegrina et al., 2020; Gammone et al., 2020; Yue-Chun et al., 2016). To explain these findings it was originally proposed that the decrease of the vascular shear-stress associated with the drug-induced bradycardia could represent the causative link between ivabradine treatment and the reduced inflammatory state (Speranza et al., 2012).

Our group considered that additional mechanisms may also be involved; for example, the established evidence that many ion channels play relevant functional roles in the immune response of white blood cells (Feske et al., 2015) raised the possibility that these cells also express HCN channels. To explore the presence of HCN signal in leukocytes, we therefore performed a preliminary investigation to detect the presence of HCN mRNA signals in human leukocytes (see details in the methods at the end of this section). Quantitative RT-PCR experiments, carried out on total leukocytes isolated from blood samples of 5 unrelated adult individuals (25-50 year-old), are shown in panel A of Fig. 2. This experiment detected significant mRNA levels for the HCN2 and HCN3 isoforms, with a prevalence of HCN2. HCN1 and HCN4 displayed instead negligible expression levels (Table 1 and Fig. 3). To explore further this interesting evidence, we isolated individual populations of lymphocytes and granulocytes by flow cytometry. Quantitative RT-PCR experiments confirmed the presence of robust HCN2 and HCN3 signals in lymphocytes, while in granulocytes HCN3 was the only isoform that could be reproducibly identified. Although preliminary, these data demonstrate the presence of isoform-specific mRNA signals in selected immune cell populations, thus suggesting a possible functional role of the channels in these cells. Even though these studies did not address the presence of HCN proteins, they open the possibility that the immuno-modulation exerted by ivabradine may also depend on the inhibition of HCN channels eventually expressed in lymphocytes. Indeed, several types of ion channels have already been described in leukocytes where they play fundamental roles in controlling cell membrane potentials and Ca^{2+} influx leading to modulation of gene expression and inflammatory responses (Grune et al., 2021). In line with this evidence Pillozzi et al. (2016) reported that hHERG inhibition in acute leukemia lymphoid cells exerts an antileukemic effect. The presence a tight crosstalk between the heart and circulating leukocytes is a well-established concept that has been investigated also at the molecular level. For example, studies on G-protein-coupled receptors and connexins 40 and 43 have shown that the oscillation in the expression of these proteins associated with cardiac dysfunctions are mirrored by similar changes in peripheral leukocytes (Lazzerini et al., 2019; Schiattarella et al., 2015). Further expression and functional studies must carefully and convincingly address the biological role of HCN in leukocytes and eventually explore the existence of a correlation with HCN expression in cardiac cells.

Methods: Blood samples were treated to induce lysis of red blood cells, and then centrifuged for 5 min at 200×*g* to precipitate

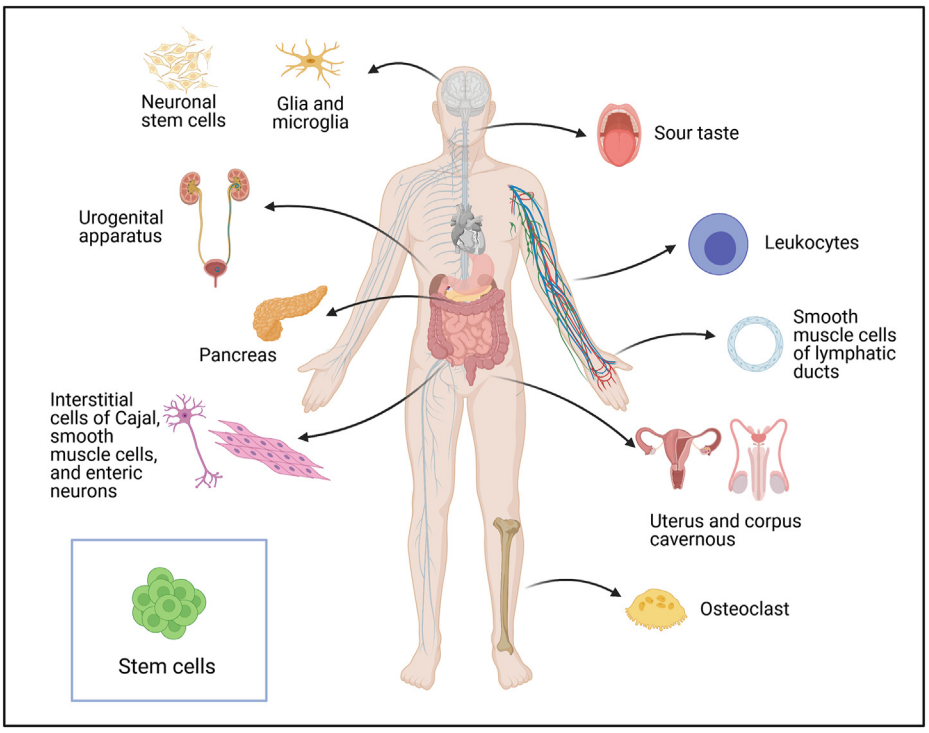

Fig. 4. *Cartoon of "uncanonical" expression pattern of HCN channels. The body districts where HCN channels display canonical functions (heart and central/peripheral nervous systems) are shown in gray, while colored insets are used to identify the sites of uncanonical functions described in this review. This scheme is based on data obtained both in human and/or animal models. "Created with BioRender.com".*

leukocytes. The cell pellet was then resuspended in appropriate buffer (Phosphate-Buffered Saline 1X (PBS) + FBS 10% + EDTA 5 mM) prior to isolating lymphocytes and granulocytes by flow cytometry analysis (FACSAria II BD Biosciences) on the basis of their cellular dimensions (forward scatter) and cytoplasmic complexity (side scatter). RNA was extracted both from single populations and from total leukocites and treated with DNASe (DNase I/RNase free, Thermo Scientific). A commercially available kit (Maxima First Strand cDNA Synthesis Kit, Thermo Scientific) was used to synthesize cDNA, and quantitative RT-PCRs were carried out with SYBR Green kit (Thermo Fisher Scientific). α-tubulin was used as the housekeeping gene to normalize the results of the genes of interest (HCN), according to the ΔCt method. Specific primers were designed using Primer-blast software. Statistical analysis (Student's T-test) was performed using OriginPro 2020. Experiments were approved by the Ethical Committee of the University of Milan.

14. Conclusions

The data presented in this review clearly illustrate that the presence of the I_f/HCN current is not limited to the heart and nervous system, but a variety of additional excitable and non-excitable cells also express these channels (Fig. 4). In some cases, the physiological functions of the I_f current are well-established, while in others they are still partly obscure due to contradictory or still unclear data. For example, many studies use I_f inhibitors to prove/disprove the role of HCN channels, but in several cases the concentrations used raise serious concern since at inappropriate (i.e. high) doses they lose their selectivity for the I_f current and may elicit unspecific effects on other ionic currents. However, the wide distribution of these channels in mammalian tissues convincingly indicates that their identification as "pacemaker" channels proposed at the beginning of their journey is just one aspect of their complex contribution to "electrical beauty" of many cell types.

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Conflict of interest

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Patrizia Benzoni: Writing – review & editing. **Giorgia Bertoli:** Writing – review & editing. **Federica Giannetti:** Writing – review & editing. **Chiara Piantoni:** Investigation. **Raffaella Milanesi:** Investigation. **Matteo Pecchiari:** Investigation. **Andrea Barbuti:** Conceptualization, Writing – review & editing. **Mirko Baruscotti:** Conceptualization, Writing – review & editing. **Annalisa Bucchi:** Conceptualization, Writing – review & editing.

Declaration of competing interest

None.

References

Agoston, A., Kunz, L., Krieger, A., Mayerhofer, A., 2004. Two types of calcium channels in human ovarian endocrine cells: involvement in steroidogenesis. J. Clin. Endocrinol. Metab. 89, 4503–4512. https://doi.org/10.1210/jc.2003-032219.

Aguilar, H.N., Mitchell, B.F., 2010. Physiological pathways and molecular mechanisms regulating uterine contractility. Hum. Reprod. Update 16, 725–744. https://doi.org/10.1093/humupd/dmq016.

Al-Naggar, I.M., Hardy, C.C., Taweh, O.G., Grabauskas, T., Mulkey, D.K., Kuchel, G.A., Smith, P.P., 2019. HCN as a mediator of urinary homeostasis: age-associated changes in expression and function in adrenergic detrusor relaxation.

Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 74, 325–329. https://doi.org/10.1093/gerona/gly137.
Allen, J.M., McHale, N.G., 1988. The effect of known K +-channel blockers on the electrical activity of bovine lymphatic smooth muscle. Pflugers Arch. Eur. J. Physiol. 411, 167–172.
Alotaibi, M., Kahlat, K., Nedjadi, T., Djouhri, L., 2017. Effects of ZD7288, a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker, on term-pregnant rat uterine contractility in vitro. Theriogenology 90, 141–146. https://doi.org/10.1016/j.theriogenology.2016.11.022.
Aquila, G., Morelli, M.B., Vieceli Dalla Sega, F., Fortini, F., Nigro, P., Caliceti, C., Ferracin, M., Negrini, M., Pannuti, A., Bonora, M., Pinton, P., Ferrari, R., Rizzo, P., 2018. Heart rate reduction with ivabradine in the early phase of atherosclerosis is protective in the endothelium of ApoE-deficient mice. J. Physiol. Pharmacol. 69, 35–52. https://doi.org/10.26402/jpp.2018.1.04.
Aydin, H.R., Turgut, H., Kurt, A., Sahan, R., Kalkan, O.F., Eren, H., Ayar, A., 2018. Ivabradine inhibits carbachol-induced contractions of isolated rat urinary bladder. Adv. Clin. Exp. Med. 27, 893–897. https://doi.org/10.17219/acem/71197.
Barbuti, A., Terragni, B., Brioschi, C., DiFrancesco, D., 2007. Localization of f-channels to caveolae mediates specific β2-adrenergic receptor modulation of rate in sinoatrial myocytes. J. Mol. Cell. Cardiol. 42, 71–78. https://doi.org/10.1016/j.yjmcc.2006.09.018.
Barbuti, A., Crespi, A., Capilupo, D., Mazzocchi, N., Baruscotti, M., DiFrancesco, D., 2009. Molecular composition and functional properties of f-channels in murine embryonic stem cell-derived pacemaker cells. J. Mol. Cell. Cardiol. 46, 343–351. https://doi.org/10.1016/j.yjmcc.2008.12.001.
Barbuti, A., Scavone, A., Mazzocchi, N., Terragni, B., Baruscotti, M., DiFrancesco, D., 2012. A caveolin-binding domain in the HCN4 channels mediates functional interaction with caveolin proteins. J. Mol. Cell. Cardiol. 53, 187–195. https://doi.org/10.1016/j.yjmcc.2012.05.013.
Baruscotti, M., Bucchi, A., DiFrancesco, D., 2005. Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacol. Ther. 107, 59–79. https://doi.org/10.1016/j.pharmthera.2005.01.005.
Baumhäkel, M., Custodis, F., Schlimmer, N., Laufs, U., Böhm, M., 2010. Heart rate reduction with ivabradine improves erectile dysfunction in parallel to decrease in atherosclerotic plaque load in ApoE-knockout mice. Atherosclerosis 212, 55–62. https://doi.org/10.1016/j.atherosclerosis.2010.03.002.
Benham, C.D., Bolton, T.B., Denbigh, J.S., Lang, R.J., 1987. Inward rectification in freshly isolated single smooth muscle cells of the rabbit jejunum. J. Physiol. 383, 461–476. https://doi.org/10.1113/jphysiol.1987.sp016421.
Biel, M., Wahl-Schott, C., Michalakis, S., Zong, X., 2009. Hyperpolarization-activated cation channels: from genes to function. Physiol. Rev. 89, 847–885. https://doi.org/10.1152/physrev.00029.2008.
Bois, P., Bescond, J., Renaudon, B., Lenfant, J., 1996. Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. Br. J. Pharmacol. 118, 1051–1057. https://doi.org/10.1111/j.1476-5381.1996.tb15505.x.
Bolívar, J.J., Tapia, D., Arenas, G., Castañón-Arreola, M., Torres, H., Galarraga, E., 2008. A hyperpolarization-activated, cyclic nucleotide-gated, (I h-like) cationic current and HCN gene expression in renal inner medullary collecting duct cells. Am. J. Physiol. Cell Physiol. 294, 893–906. https://doi.org/10.1152/ajpcell.00616.2006.
Bonadei, I., Sciatti, E., Vizzardi, E., Fabbricatore, D., Pagnoni, M., Rossi, L., Carubelli, V., Lombardi, C.M., Metra, M., 2018. Effects of ivabradine on endothelial function, aortic properties and ventricular-arterial coupling in chronic systolic heart failure patients. Cardiovasc. Ther. 36, e12323. https://doi.org/10.1111/1755-5922.12323.
BoSmith, R.E., Briggs, I., Sturgess, N.C., 1993. Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (If) in Guinea-pig dissociated sinoatrial node cells. Br. J. Pharmacol. 110, 343–349.
Boyle, W.J., Simonet, W.S., Lacey, D.L., 2003. Osteoclast differentiation and activation. Nature 423, 337–342. https://doi.org/10.1038/nature01658.
Brown, H.F., DiFranceso, D., Noble, S.J., 1979. How does adrenaline accelerate the heart? Nature 280, 235–236. https://doi.org/10.1038/280235a0.
Bucchi, A., Barbuti, A., Baruscotti, M., DiFrancesco, D., 2007. Heart rate reduction via selective "funny" channel blockers. Curr. Opin. Pharmacol. https://doi.org/10.1016/j.coph.2006.09.005.
Calejo, Ana I., Jorgačevski, J., Rituper, B., Guček, A., Pereira, P.M., Santos, M.A.S., Potokar, M., Vardjan, N., Kreft, M., Gonçalves, P.P., Zorec, R., 2014. Hyperpolarization-activated cyclic nucleotide-gated channels and cAMP-dependent modulation of exocytosis in cultured rat lactotrophs. J. Neurosci. 34, 15638–15647. https://doi.org/10.1523/JNEUROSCI.5290-13.2014.
Calejo, Ana I., Reverendo, M., Silva, V.S., Pereira, P.M., Santos, M.A.S., Zorec, R., Gonçalves, P.P., 2014a. Differences in the expression pattern of HCN isoforms among mammalian tissues: sources and implications. Mol. Biol. Rep. 41, 297–307. https://doi.org/10.1007/s11033-013-2862-2.
Carmeliet, E., 2019. Pacemaking in cardiac tissue. From Ik2 to a coupled-clock system. Phys. Rep. https://doi.org/10.14814/phy2.13862.
Carrisoza-Gaytán, R., Rangel, C., Salvador, C., Saldaña-Meyer, R., Escalona, C., Satlin, L.M., Liu, W., Zavilowitz, B., Trujillo, J., Bobadilla, N.A., Escobar, L.I., 2011. The hyperpolarization-activated cyclic nucleotide-gated HCN2 channel transports ammonium in the distal nephron. Kidney Int. 80, 832–840. https://doi.org/10.1038/ki.2011.230.
Chu, Z., Takagi, H., Moenter, S.M., 2010. Hyperpolarization-activated currents in Gonadotropin-Releasing Hormone (GnRH) neurons contribute to intrinsic excitability and are regulated by gonadal steroid feedback. J. Neurosci. 30, 13373–13383. https://doi.org/10.1523/JNEUROSCI.1687-10.2010.
Custodis, F., Baumhäkel, M., Schlimmer, N., List, F., Gensch, C., Böhm, M., Laufs, U., 2008. Heart rate reduction by ivabradine reduces oxidative stress, improves endothelial function, and prevents atherosclerosis in apolipoprotein E-deficient mice. Circulation 117, 2377–2387. https://doi.org/10.1161/CIRCULATIONAHA.107.746537.
Dallapellegrina, L., Sciatti, E., Vizzardi, E., 2020. Ivabradine and endothelium: an update. Ther. Adv. Cardiovasc. Dis. 14. https://doi.org/10.1177/1753944720934937, 1753944720934937.
Delpón, E., Valenzuela, C., Pérez, O., Franqueza, L., Gay, P., Snyders, D.J., Tamargo, J., 1996. Mechanisms of block of a human cloned potassium channel by the enantiomers of a new bradycardic agent: S-16257-2 and S-16260-2. Br. J. Pharmacol. 117, 1293–1301. https://doi.org/10.1111/j.1476-5381.1996.tb16728.x.
Demontis, G.C., Gargini, C., Paoli, T.G., Cervetto, L., 2009. Selective Hcn1 channels inhibition by ivabradine in mouse rod photoreceptors. Invest. Ophthalmol. Vis. Sci. 50. https://doi.org/10.1167/iovs.08-2659, 1948–55.
Deng, T., Zhang, Q., Wang, Q., Zhong, X., Li, L., 2015. Changes in hyperpolarization-activated cyclic nucleotide-gated channel expression and activity in bladder interstitial cells of Cajal from rats with detrusor overactivity. Int. UrogynEcol. J. Pelvic Floor Dysfunct. 26, 1139–1145. https://doi.org/10.1007/s00192-015-2632-x.
Denyer, J.C., Brown, H.F., 1990. Pacemaking in rabbit isolated sino-atrial node cells during Cs+ block of the hyperpolarization-activated current if. J. Physiol. 429, 401–409. https://doi.org/10.1113/jphysiol.1990.sp018264.
DiFrancesco, D., 1981. A new interpretation of the pace-maker current in calf Purkinje fibres. J. Physiol. 314, 359–376. https://doi.org/10.1113/jphysiol.1981.sp013713.
DiFrancesco, D., 1982. Block and activation of the pace-maker channel in calf purkinje fibres: effects of potassium, caesium and rubidium. J. Physiol. 329, 485–507. https://doi.org/10.1113/jphysiol.1982.sp014315.
DiFrancesco, D., 2020. A brief history of pacemaking. Front. Physiol.
Dong, X., Song, Q., Zhu, J., Zhao, J., Liu, Q., Zhang, T., Long, Z., Li, J., Wu, C., Wang, Q., Hu, X., Damaser, M., Li, L., 2016. Interaction of Caveolin-3 and HCN is involved in the pathogenesis of diabetic cystopathy. Sci. Rep. 6, 1–14. https://doi.org/10.1038/srep24844.
Dorrell, C., Schug, J., Canaday, P.S., Russ, H.A., Tarlow, B.D., Grompe, M.T., Horton, T., Hebrok, M., Streeter, P.R., Kaestner, K.H., Grompe, M., 2016. Human islets contain four distinct subtypes of β cells. Nat. Commun. 7, 1–9. https://doi.org/10.1038/ncomms11756.
El-Kholy, W., MacDonald, P.E., Fox, J.M., Bhattacharjee, A., Xue, T., Gao, X., Zhang, Y., Stieber, J., Li, R.A., Tsushima, R.G., Wheeler, M.B., 2007. Hyperpolarization-activated cyclic nucleotide-gated channels in pancreatic β-cell. Mol. Endocrinol. 21, 753–764. https://doi.org/10.1210/me.2006-0258.
Feske, S., Wulff, H., Skolnik, E.Y., 2015. Ion channels in innate and adaptive immunity. Annu. Rev. Immunol. 33, 291–353. https://doi.org/10.1146/annurev-immunol-032414-112212.
Fletcher, P.A., Sherman, A., Stojilkovic, S.S., 2018. Common and diverse elements of ion channels and receptors underlying electrical activity in endocrine pituitary cells. Mol. Cell. Endocrinol. 463, 23–36. https://doi.org/10.1016/j.mce.2017.06.022.
Fujii, K., Nakajo, K., Egashira, Y., Yamamoto, Y., Kitada, K., Taniguchi, K., Kawai, M., Tomiyama, H., Kawakami, K., Uchiyama, K., Ono, F., 2020. Gastrointestinal neurons expressing HCN4 regulate retrograde peristalsis. Cell Rep. 30, 2879–2888. https://doi.org/10.1016/j.celrep.2020.02.024 e3.
Galligan, J.J., Tatsumi, H., Shen, K.Z., Surprenant, A., North, R.A., 1990. Cation current activated by hyperpolarization (IH) in Guinea pig enteric neurons. Am. J. Physiol. Liver Physiol. 259, G966–G972. https://doi.org/10.1152/ajpgi.1990.259.6.G966.
Gammone, M.A., Riccioni, G., Massari, F., D'Orazio, N., 2020. Beneficial effect of ivabradine against cardiovascular diseases. Front. Biosci. 12, 161–172.
Gasparini, S., DiFrancesco, D., 1997. Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. Pflügers Archiv 435, 99–106. https://doi.org/10.1007/s004240050488.
Gauss, R., Seifert, R., Kaupp, U.B., 1998. Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. Nature 393, 583–587. https://doi.org/10.1038/31248.
Giannetti, F., Benzoni, P., Campostrini, G., Milanesi, R., Bucchi, A., Baruscotti, M., Dell'Era, P., Rossini, A., Barbuti, A., 2021. A detailed characterization of the hyperpolarization-activated "funny" current (If) in human-induced pluripotent stem cell (iPSC)-derived cardiomyocytes with pacemaker activity. Pflügers Archiv. https://doi.org/10.1007/s00424-021-02571-w.
Gonzalez-Iglesias, A.E., Kretschmannova, K., Tomic, M., Stojilkovic, S.S., 2006. ZD7288 inhibits exocytosis in an HCN-independent manner and downstream of voltage-gated calcium influx in pituitary lactotrophs. Biochem. Biophys. Res. Commun. 346, 845–850. https://doi.org/10.1016/j.bbrc.2006.05.194.
Green, M.E., Edwards, G., Kirkup, A.J., Miller, M., Weston, A.H., 1996. Pharmacological characterization of the inwardly-rectifying current in the smooth muscle cells of the rat bladder. Br. J. Pharmacol. 119, 1509.
Greenwood, I.A., Prestwich, S.A., 2002. Characteristics of hyperpolarization-activated cation currents in portal vein smooth muscle cells. Am. J. Physiol. Physiol. 282, C744–C753.
Grune, J., Yamazoe, M., Nahrendorf, M., 2021. Electroimmunology and cardiac arrhythmia. Nat. Rev. Cardiol. https://doi.org/10.1038/s41569-021-00520-9.
Guatteo, E., Stanness, K.A., Janigro, D., 1996. Hyperpolarization-activated ion currents in cultured rat cortical and spinal cord astrocytes. Glia 16, 196–209. https://doi.org/10.1002/(SICI)1098-1136(199603)16:3<196::AID-

GLIA2>3.0.CO;2-0.
Guo, T., Li, Jiade, Li, Jing, Kong, D., Bi, C., He, Z., Tang, D., Jin, X., 2017. Association between hyperpolarization-activated channel in interstitial cells of cajal and gastrointestinal dysmotility induced by malignant ascites. Oncol. Lett. 13, 1601–1608. https://doi.org/10.3892/ol.2017.5652.
Gur, S., Alzweri, L., Yilmaz-Oral, D., Kaya-Sezginer, E., Abdel-Mageed, A.B., Sikka, S.C., Hellstrom, W.J.G., 2019. Ivabradine, the hyperpolarization-activated cyclic nucleotide-gated channel blocker, elicits relaxation of the human corpus cavernosum: a potential option for erectile dysfunction treatment. Aging Male 1–10. https://doi.org/10.1080/13685538.2019.1678125, 0.
Haechl, N., Ebner, J., Hilber, K., Todt, H., Koenig, X., 2019. Pharmacological profile of the bradycardic agent ivabradine on human cardiac ion channels. Cell. Physiol. Biochem. 53, 36–48. https://doi.org/10.33594/000000119.
Harris, N.C., Constanti, A., 1995. Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in Guinea pig substantia nigra neurons in vitro. J. Neurophysiol. 74, 2366–2378. https://doi.org/10.1152/jn.1995.74.6.2366.
Hashitani, H., Nguyen, M.J., Noda, H., Mitsui, R., Higashi, R., Ohta, K., Nakamura, K.I., Lang, R.J., 2017. Interstitial cell modulation of pyeloureteric peristalsis in the mouse renal pelvis examined using FIBSEM tomography and calcium indicators. Pflugers Arch. Eur. J. Physiol. 469, 797–813. https://doi.org/10.1007/s00424-016-1930-6.
He, P., Deng, J., Zhong, X., Zhou, Z., Song, B., Li, L., 2012. Identification of a hyperpolarization-activated cyclic nucleotide-gated channel and its subtypes in the urinary bladder of the rat. Urology 79, 1411. https://doi.org/10.1016/j.urology.2012.01.037 e7-1411.e13.
He, F., Yang, Z., Dong, X., Fang, Z., Liu, Q., Hu, X., Yi, S., Li, L., 2018. The role of HCN channels in peristaltic dysfunction in human ureteral tuberculosis. Int. Urol. Nephrol. 50, 639–645. https://doi.org/10.1007/s11255-018-1816-y.
Honsa, P., Pivonkova, H., Harantova, L., Butenko, O., Kriska, J., Dzamba, D., Rusnakova, V., Valihrach, L., Kubista, M., Anderova, M., 2014. Increased expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in reactive astrocytes following ischemia. Glia 62, 2004–2021. https://doi.org/10.1002/glia.22721.
Hsiao, H.-T., Liu, Y.-C., Liu, P.-Y., Wu, S.-N., 2019. Concerted suppression of Ih and activation of Ik(M) by ivabradine, an HCN-channel inhibitor, in pituitary cells and hippocampal neurons. Brain Res. Bull. 149, 11–20. https://doi.org/10.1016/j.brainresbull.2019.03.016.
Hurtado, R., Bub, G., Herzlinger, D., 2010. The pelvis–kidney junction contains HCN3, a hyperpolarization-activated cation channel that triggers ureter peristalsis. Kidney Int. 77, 500–508. https://doi.org/10.1038/ki.2009.483.
Hurtado, R., Bub, G., Herzlinger, D., 2014. A molecular signature of tissues with pacemaker activity in the heart and upper urinary tract involves coexpressed hyperpolarization-activated cation and T-type Ca2+ channels. Faseb. J. 28, 730–739. https://doi.org/10.1096/fj.13-237289.
Ili, C., Buchegger, K., Demond, H., Castillo-Fernandez, J., Kelsey, G., Zanella, L., Abanto, M., Riquelme, I., López, J., Viscarra, T., García, P., Bellolio, E., Saavedra, D., Brebi, P., 2020. Landscape of genome-wide dna methylation of colorectal cancer metastasis. Cancers 12, 1–20. https://doi.org/10.3390/cancers12092710.
Izquierdo, P., Attwell, D., Madry, C., 2019. Ion channels and receptors as determinants of microglial function. Trends Neurosci. 42, 278–292. https://doi.org/10.1016/j.tins.2018.12.007.
Johard, H., Omelyanenko, A., Fei, G., Zilberter, M., Dave, Z., Abu-Youssef, R., Schmidt, L., Harisankar, A., Vincent, C.T., Walfridsson, J., Nelander, S., Harkany, T., Blomgren, K., Andäng, M., 2020. HCN channel activity balances quiescence and proliferation in neural stem cells and is a selective target for neuroprotection during cancer treatment. Mol. Canc. Res. 18, 1522–1533. https://doi.org/10.1158/1541-7786.mcr-20-0292.
Kashyap, M., Yoshimura, N., Smith, P.P., Chancellor, M., Tyagi, P., 2015. Characterization of the role of HCN channels in β3-adrenoceptor mediated rat bladder relaxation. Bladder 2, 15. https://doi.org/10.14440/bladder.2015.44.
Kashyap, M., Singh, N., Yoshimura, N., Chermansky, C., Tyagi, P., 2020. Constitutively active HCN channels constrain detrusor excitability and modulate evoked contractions of human bladder 8, 163–176.
Kimelberg, H.K., Nedergaard, M., 2010. Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics 7, 338–353. https://doi.org/10.1016/j.nurt.2010.07.006.
Kirkpatrick, C.T., McHale, N.G., 1977. Electrical and mechanical activity of isolated lymphatic vessels [proceedings]. J. Physiol. 272, 33P–34P.
Kobayashi, Y., Mizoguchi, T., Take, I., Kurihara, S., Udagawa, N., Takahashi, N., 2005. Prostaglandin E2 enhances osteoclastic differentiation of precursor cells through protein kinase A-dependent phosphorylation of TAK1*. J. Biol. Chem. 280, 11395–11403. https://doi.org/10.1074/jbc.M411189200.
Koncz, I., Szél, T., Bitay, M., Cerbai, E., Jaeger, K., Fülöp, F., Jost, N., Virág, L., Orvos, P., Tálosi, L., Kristóf, A., Baczkó, I., Papp, J.G., Varró, A., 2011. Electrophysiological effects of ivabradine in dog and human cardiac preparations: potential antiarrhythmic actions. Eur. J. Pharmacol. 668, 419–426. https://doi.org/10.1016/j.ejphar.2011.07.025.
Kretschmannova, K., Kucka, M., Gonzalez-Iglesias, A.E., Stojilkovic, S.S., 2012. The expression and role of hyperpolarization-activated and cyclic nucleotide-gated channels in endocrine anterior pituitary cells. Mol. Endocrinol. 26, 153–164. https://doi.org/10.1210/me.2011-1207.
Kunz, L., Thalhammer, A., Berg, F.D., Berg, U., Duffy, D.M., Stouffer, R.L., Dissen, G.A., Ojeda, S.R., Mayerhofer, A., 2002. Ca2+-Activated, large conductance K+ channel in the ovary: identification, characterization, and functional involvement in steroidogenesis. J. Clin. Endocrinol. Metab. 87, 5566–5574. https://doi.org/10.1210/jc.2002-020841.
Kunz, L., Rämsch, R., Krieger, A., Young, K.A., Dissen, G.A., Stouffer, R.L., Ojeda, S.R., Mayerhofer, A., 2006. Voltage-dependent K+ channel acts as sex steroid sensor in endocrine cells of the human ovary. J. Cell. Physiol. 206, 167–174. https://doi.org/10.1002/jcp.20453.
Lau, Y.-T., Wong, C.-K., Luo, J., Leung, L.-H., Tsang, P.-F., Bian, Z.-X., Tsang, S.-Y., 2011. Effects of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blockers on the proliferation and cell cycle progression of embryonic stem cells. Pflügers Arch. - Eur. J. Physiol. 461, 191–202. https://doi.org/10.1007/s00424-010-0899-9.
Lazzerini, P.E., Laghi-Pasini, F., Acampa, M., Srivastava, U., Bertolozzi, I., Giabbani, B., Finizola, F., Vanni, F., Dokollari, A., Natale, M., Cevenini, G., Selvi, E., Migliacci, N., Maccherini, M., Boutjdir, M., Capecchi, P.L., 2019. Systemic inflammation rapidly induces reversible atrial electrical remodeling: the role of interleukin-6–mediated changes in connexin expression. J. Am. Heart Assoc. 8. https://doi.org/10.1161/JAHA.118.011006.
Lee, M.Y., Ha, S.E., Park, C., Park, P.J., Fuchs, R., Wei, L., Jorgensen, B.G., Redelman, D., Ward, S.M., Sanders, K.M., Ro, S., 2017. Transcriptome of interstitial cells of Cajal reveals unique and selective gene signatures. PloS One 1–25. https://doi.org/10.1371/journal.pone.0176031.
León-Aparicio, D., Salvador, C., Aparicio-Trejo, O.E., Briones-Herrera, A., Pedraza-Chaverri, J., Vaca, L., Sampieri, A., Padilla-Flores, T., López-González, Z., León-Contreras, J.C., Hernández-Pando, R., Escobar, L.I., 2019. Novel potassium channels in kidney mitochondria: the hyperpolarization-activated and cyclic nucleotide-gated HCN channels. Int. J. Mol. Sci. 20, 1–18. https://doi.org/10.3390/ijms20204995.
Levin, M., 2014. Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration. J. Physiol. 592, 2295–2305. https://doi.org/10.1113/jphysiol.2014.271940.
Levin, M., Stevenson, C.G., 2012. Regulation of cell behavior and tissue patterning by bioelectrical signals: challenges and opportunities for biomedical engineering. Annu. Rev. Biomed. Eng. 14, 295–323. https://doi.org/10.1146/annurev-bioeng-071811-150114.
Li, B., Zhang, J., Wang, Z., Chen, S., 2016. Ivabradine prevents low shear stress induced endothelial inflammation and oxidative stress via mTOR/eNOS pathway. PloS One 11, e0149694.
López-González, Z., Ayala-Aguilera, C., Martinez-Morales, F., Galicia-Cruz, O., Salvador-Hernández, C., Pedraza-Chaverri, J., Medeiros, M., Hernández, A.M., Escobar, L.I., 2016. Immunolocalization of hyperpolarization-activated cationic HCN1 and HCN3 channels in the rat nephron: regulation of HCN3 by potassium diets. Histochem. Cell Biol. 145, 25–40. https://doi.org/10.1007/s00418-015-1375-6.
López-González, Z., Padilla-Flores, T., León-Aparicio, D., Gutiérrez-Vásquez, E., Salvador, C., León-Contreras, J.C., Hernández-Pando, R., Escobar, L.I., 2020. Metabolic acidosis and hyperkalemia differentially regulate cation HCN3 channel in the rat nephron. J. Mol. Histol. 51, 701–716. https://doi.org/10.1007/s10735-020-09916-2.
Lu, J.Y., Ying, X.W., Chen, X.L., Tu, W.Z., Li, S.S., Jiang, S.H., 2020. Effects of electroacupuncture at different acupoints on the histomorphology of neurogenic bladder and the expression of hyperpolarization-activated cyclic nucleotide-gated channels in interstitial cells of cajal in a rat model of suprasacral spinal cord i. Ann. Palliat. Med. 9, 3830–3838. https://doi.org/10.21037/apm-20-1827.
Mader, F., Müller, S., Krause, L., Springer, A., Kernig, K., Protzel, C., Porath, K., Rackow, S., Wittstock, T., Frank, M., Hakenberg, O.W., Köhling, R., Kirschstein, T., 2018. Hyperpolarization-activated cyclic nucleotide-gated non-selective (HCN) ion channels regulate human and murine urinary bladder contractility. Front. Physiol. 9, 753. https://doi.org/10.3389/fphys.2018.00753.
Maylie, J., Weiss, J., Morad, M., 1979. Pacemaker Currents and Extracellular K+ Activity in Rabbit Sa Node 25, Undefined-Undefined.
McCloskey, K.D., Toland, H.M., Hollywood, M.A., Thornbury, K.D., McHale, N.G., 1999. Hyperpolarisation-activated inward current in isolated sheep mesenteric lymphatic smooth muscle. J. Physiol. 521, 201–211. https://doi.org/10.1111/j.1469-7793.1999.00201.x.
McCulloh, D.H., Levitan, H., 1987. Rabbit oocyte maturation: changes of membrane resistance, capacitance, and the frequency of spontaneous transient depolarizations. Dev. Biol. 120, 162–169. https://doi.org/10.1016/0012-1606(87)90114-X.
Mert, K.U., Dural, M., Mert, G.Ö., Iskenderov, K., Özen, A., 2018. Effects of heart rate reduction with ivabradine on the international ındex of erectile function (IIEF-5) in patients with heart failure. Aging Male 21, 93–98. https://doi.org/10.1080/13685538.2017.1369943.
Messenger, J.P., Bornstein, J.C., Furness, J.B., 1994. Electrophysiological and morphological classification of myenteric neurons in the proximal colon of the Guinea-pig. Neuroscience 60, 227–244. https://doi.org/10.1016/0306-4522(94)90217-8.
Michels, G., Brandt, M.C., Zagidullin, N., Khan, I.F., Larbig, R., Van Aaken, S., Wippermann, J., Hoppe, U.C., 2008. Direct evidence for calcium conductance of hyperpolarization-activated cyclic nucleotide-gated channels and human native if at physiological calcium concentrations. Cardiovasc. Res. 78, 466–475. https://doi.org/10.1093/cvr/cvn032.
Negrini, D., Marcozzi, C., Solari, E., Bossi, E., Cinquetti, R., Reguzzoni, M., Moriondo, A., 2016. Hyperpolarization-activated cyclic nucleotide-gated channels in peripheral diaphragmatic lymphatics. Am. J. Physiol. Heart Circ. Physiol. 311. https://doi.org/10.1152/ajpheart.00193.2016. H892–H903.

Ng, S.-Y., Chin, C.-H., Lau, Y.-T., Luo, J., Wong, C.-K., Bian, Z.-X., Tsang, S.-Y., 2010. Role of voltage-gated potassium channels in the fate determination of embryonic stem cells. J. Cell. Physiol. 224, 165–177. https://doi.org/10.1002/jcp.22113.

Noble, D., 1960. Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations. Nature 188, 495–497. https://doi.org/10.1038/188495b0.

Noma, A., Irisawa, H., 1976. Membrane currents in the rabbit sinoatrial node cell as studied by the double microelectrode method. Pflügers Arch. Eur. J. Physiol. 364, 45–52. https://doi.org/10.1007/BF01062910.

Norberg, E., Karlsson, M., Korenovska, O., Szydlowski, S., Silberberg, G., Uhlén, P., Orrenius, S., Zhivotovsky, B., 2010. Critical role for hyperpolarization-activated cyclic nucleotide-gated channel 2 in the AIF-mediated apoptosis. EMBO J. 29, 3869–3878. https://doi.org/10.1038/emboj.2010.253.

Notomi, T., Kuno, M., Hiyama, A., Ohura, K., Noda, M., Skerry, T.M., 2015. Zinc-induced effects on osteoclastogenesis involves activation of hyperpolarization-activated cyclic nucleotide modulated channels via changes in membrane potential. J. Bone Miner. Res. 30, 1618–1626. https://doi.org/10.1002/jbmr.2507.

Okabe, K., Kajiya, H., Okamoto, F., Soeda, H., Inoue, Y., Kawarabayashi, T., 1999. Physiological significance of hyperpolarization-activated inward currents (I(h)) in smooth muscle cells from the circular layers of pregnant rat myometrium. Pflugers Arch. Eur. J. Physiol. 439, 76–85. https://doi.org/10.1007/s004240051130.

Omelyanenko, A., Sekyrova, P., Andäng, M., 2016. ZD7288, a blocker of the HCN channel family, increases doubling time of mouse embryonic stem cells and modulates differentiation outcomes in a context-dependent manner. SpringerPlus 5, 1–10. https://doi.org/10.1186/s40064-016-1678-7.

O'Donnell, A.M., Coyle, D., Puri, P., 2015. Decreased expression of hyperpolarisation-activated cyclic nucleotide-gated channel 3 in Hirschsprung's disease. World J. Gastroenterol. 21, 5635–5640. https://doi.org/10.3748/wjg.v21.i18.5635.

Padilla-Flores, T., López-González, Z., Vaca, L., Aparicio-Trejo, O.E., Briones-Herrera, A., Riveros-Rosas, H., Pedraza-Chaverri, J., León-Aparicio, D., Salvador, C., Sampieri, A., Escobar, L.I., 2020. “Funny” channels in cardiac mitochondria modulate membrane potential and oxygen consumption. Biochem. Biophys. Res. Commun. 524, 1030–1036. https://doi.org/10.1016/j.bbrc.2020.02.033.

Pai, V.P., Willocq, V., Pitcairn, E.J., Lemire, J.M., Paré, J.-F., Shi, N.-Q., McLaughlin, K.A., Levin, M., 2017. HCN4 ion channel function is required for early events that regulate anatomical left-right patterning in a nodal and lefty asymmetric gene expression-independent manner. Biol. Open 6, 1445. https://doi.org/10.1242/bio.025957. LP – 1457.

Pape, H.-C., McCormick, D.A., 1989. Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. Nature 340, 715–718. https://doi.org/10.1038/340715a0.

Park, J.H., Kim, D.W., Lee, T.K., Park, C.W., Park, Y.E., Ahn, J.H., Lee, H.A., Won, M.H., Lee, C.H., 2019. Improved HCN channels in pyramidal neurons and their new expression levels in pericytes and astrocytes in the gerbil hippocampal CA1 subfield following transient ischemia. Int. J. Mol. Med. 44, 1801–1810. https://doi.org/10.3892/ijmm.2019.4353.

Pian, P., Bucchi, A., DeCostanzo, A., Robinson, R.B., Siegelbaum, S.A., 2007. Modulation of cyclic nucleotide-regulated HCN channels by PIP2 and receptors coupled to phospholipase C. Pflugers Arch. Eur. J. Physiol. https://doi.org/10.1007/s00424-007-0295-2.

Pillozzi, S., Masselli, M., Gasparoli, L., D'Amico, M., Polletta, L., Veltroni, M., Favre, C., Basso, G., Becchetti, A., Arcangeli, A., 2016. Macrolide antibiotics exert anti-leukemic effects by modulating the autophagic flux through inhibition of hERG1 potassium channels. Blood Canc. J. 6. https://doi.org/10.1038/bcj.2016.32 e423–e423.

Pitcairn, E., Harris, H., Epiney, J., Pai, V.P., Lemire, J.M., Ye, B., Shi, N.-Q., Levin, M., McLaughlin, K.A., 2017. Coordinating heart morphogenesis: a novel role for hyperpolarization-activated cyclic nucleotide-gated (HCN) channels during cardiogenesis in Xenopus laevis. Commun. Integr. Biol. 10. https://doi.org/10.1080/19420889.2017.1309488 e1309488–e1309488.

Platano, D., Magli, M.C., Ferraretti, A.P., Gianaroli, L., Aicardi, G., 2005. L- and T-type voltage-gated Ca2+ channels in human granulosa cells: functional characterization and cholinergic regulation. J. Clin. Endocrinol. Metab. 90, 2192–2197. https://doi.org/10.1210/jc.2004-1819.

Robinson, R.B., Siegelbaum, S.A., 2003. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu. Rev. Physiol. 65, 453–480. https://doi.org/10.1146/annurev.physiol.65.092101.142734.

Rusnakova, V., Honsa, P., Dzamba, D., Ståhlberg, A., Kubista, M., Anderova, M., 2013. Heterogeneity of astrocytes: from development to injury - single cell gene expression. PloS One 8. https://doi.org/10.1371/journal.pone.0069734.

Sánchez-Alonso, J.L., Halliwell, J.V., Colino, A., 2008. ZD 7288 inhibits T-type calcium current in rat hippocampal pyramidal cells. Neurosci. Lett. 439, 275–280. https://doi.org/10.1016/j.neulet.2008.05.016.

Sanders, K.M., Ward, S.M., Koh, S.D., 2014. Interstitial cells: regulators of smooth muscle function. Physiol. Rev. 94, 859–907. https://doi.org/10.1152/physrev.00037.2013.

Santoro, B., Shah, M.M., 2020. Hyperpolarization-activated cyclic nucleotide-gated channels as drug targets for neurological disorders. Annu. Rev. Pharmacol. Toxicol. 60, 109–131. https://doi.org/10.1146/annurev-pharmtox-010919-023356.

Sartiani, L., Bettioli, E., Stillitano, F., Mugelli, A., Cerbai, E., Jaconi, M.E., 2007. Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. Stem Cell. 25, 1136–1144. https://doi.org/10.1634/stemcells.2006-0466.

Satoh, H., 1995. Identification of a hyperpolarization-activated inward current in uterine smooth muscle cells during pregnancy. Gen. Pharmacol. 26, 1335–1338. https://doi.org/10.1016/0306-3623(95)00006-M.

Satoh, T.-O., Yamada, M., 2000. A bradycardiac agent ZD7288 blocks the hyperpolarization-activated current (Ih) in retinal rod photoreceptors. Neuropharmacology 39, 1284–1291. https://doi.org/10.1016/S0028-3908(99)00207-5.

Schiattarella, G.G., Magliulo, F., Cattaneo, F., Gargiulo, G., Sannino, A., Franzone, A., Oliveti, M., Perrino, C., Trimarco, B., Esposito, G., 2015. Novel molecular approaches in heart failure: seven trans-membrane receptors signaling in the heart and circulating blood leukocytes. Front. Cardiovasc. Med. 2. https://doi.org/10.3389/fcvm.2015.00013.

Seo, H., Seol, M.J., Lee, K., 2015. Differential expression of hyperpolarization-activated cyclic nucleotide-gated channel subunits during hippocampal development in the mouse. Mol. Brain 8, 1–14. https://doi.org/10.1186/s13041-015-0103-4.

Seyama, I., 1976. Characteristics of the rectifying properties of the sino-atrial node cell of the rabbit. J. Phy~iol.

Shahi, P.K., Choi, S., Zuo, D.C., Kim, M.Y., Park, C.G., Kim, Y.D., Lee, J., Park, K.J., So, I., Jun, J.Y., 2014. The possible roles of hyperpolarization-activated cyclic nucleotide channels in regulating pacemaker activity in colonic interstitial cells of Cajal. J. Gastroenterol. 49, 1001–1010. https://doi.org/10.1007/s00535-013-0849-3.

Shen, H., Liu, W., Geng, Q., Li, H., Lu, M., Liang, P., Zhang, B., Yamoah, E.N., Lv, P., 2018. Age-dependent up-regulation of HCN channels in spiral ganglion neurons coincide with hearing loss in mice. Front. Aging Neurosci. 10, 1–11. https://doi.org/10.3389/fnagi.2018.00353.

Si, X., Huang, L., Gong, Y., Lu, J., Lin, L., 2012. Role of calcium in activation of hyperpolarization-activated cyclic nucleotide-gated channels caused by cholecystokinin octapeptide in interstitial cells of cajal. Digestion 85, 266–275. https://doi.org/10.1159/000337077.

Simasko, S.M., Sankaranarayanan, S., 1997. Characterization of a hyperpolarization-activated cation current in rat pituitary cells. Am. J. Physiol. Metab. 272, E405–E414. https://doi.org/10.1152/ajpendo.1997.272.3.E405.

Speranza, L., Franceschelli, S., Riccioni, G., 2012. The biological effects of ivabradine in cardiovascular disease. Molecules 17, 4924–4935. https://doi.org/10.3390/molecules17054924.

Stevens, D.R., Seifert, R., Bufe, B., Müller, F., Kremmer, E., Gauss, R., Meyerhof, W., Kaupp, U.B., Lindemann, B., 2001. Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature 413, 631–635. https://doi.org/10.1038/35098087.

Stieber, J., Wieland, K., Stöckl, G., Ludwig, A., Hofmann, F., 2006. Bradycardic and proarrhythmic properties of sinus node inhibitors. Mol. Pharmacol. 69, 1328–1337. https://doi.org/10.1124/mol.105.020701.

Sundelacruz, S., Levin, M., Kaplan, D.L., 2009. Role of membrane potential in the regulation of cell proliferation and differentiation. Stem Cell Rev. Reports 5, 231–246. https://doi.org/10.1007/s12015-009-9080-2.

Telinius, N., Majgaard, J., Kim, S., Katballe, N., Pahle, E., Nielsen, J., Hjortdal, V., Aalkjaer, C., Boedtkjer, D.B., 2015. Voltage-gated sodium channels contribute to action potentials and spontaneous contractility in isolated human lymphatic vessels. J. Physiol. 593, 3109–3122. https://doi.org/10.1113/JP270166.

Uawithya, P., Pisitkun, T., Ruttenberg, B.E., Knepper, M.A., 2008. Transcriptional profiling of native inner medullary collecting duct cells from rat kidney. Physiol. Genom. 32, 229–253. https://doi.org/10.1152/physiolgenomics.00201.2007.

Vay, S.U., Flitsch, L.J., Rabenstein, M., Monière, H., Jakovcevski, I., Andjus, P., Bijelic, D., Blaschke, S., Walter, H.L., Fink, G.R., Schroeter, M., Rueger, M.A., 2020. The impact of hyperpolarization-activated cyclic nucleotide-gated (HCN) and voltage-gated potassium KCNQ/Kv7 channels on primary microglia function. J. Neuroinflammation 17, 1–17. https://doi.org/10.1186/s12974-020-01779-4.

Walcher, T., Bernhardt, P., Vasic, D., Bach, H., Durst, R., Rottbauer, W., Walcher, D., 2010. Ivabradine reduces chemokine-induced CD4-positive lymphocyte migration. Mediat. Inflamm. 751313. https://doi.org/10.1155/2010/751313, 2010.

Wang, K., Xue, T., Tsang, S.-Y., Van Huizen, R., Wong, C.W., Lai, K.W., Ye, Z., Cheng, L., Au, K.W., Zhang, J., Li, G.-R., Lau, C.-P., Tse, H.-F., Li, R.A., 2005. Electrophysiological properties of pluripotent human and mouse embryonic stem cells. Stem Cell. 23, 1526–1534. https://doi.org/10.1634/stemcells.2004-0299.

Ward, S.M., Sanders, K.M., Thornbury, K.D., McHale, N.G., 1991. Spontaneous electrical-activity in isolated bovine lymphatics recorded by intracellular microelectrodes. In: JOURNAL OF PHYSIOLOGY-LONDON. CAMBRIDGE UNIV PRESS 40 WEST 20TH STREET, NEW YORK, NY, 10011-4211, P168–P168.

Weidmann, S., 1951. Effect of current flow on the membrane potential of cardiac muscle. J. Physiol. 115, 227–236. https://doi.org/10.1113/jphysiol.1951.sp004667.

Weiss, J., Maylie, J., Morad, M., 1978. Pacemaker currents and paracellular K+ accumulation in rabbit sinoatrial node. In: Frontiers of Biological Energetics. Elsevier, pp. 1417–1426. https://doi.org/10.1016/b978-0-12-225402-4.50079-6.

Wray, S., Prendergast, C., 2019. The myometrium: from excitation to contractions and labour. Advances in Experimental Medicine and Biology. https://doi.org/10.1007/978-981-13-5895-1_10.

Wu, X., Liao, L., Liu, X., Luo, F., Yang, T., Li, C., 2012. Is ZD7288 a selective blocker of hyperpolarization-activated cyclic nucleotide-gated channel currents? Channels 6, 438–442. https://doi.org/10.4161/chan.22209.

Wu, C., Dong, X., Liu, Q., Zhu, J., Zhang, T., Wang, Q., Hu, X., Yang, Z., Li, L., 2017. EP3 activation facilitates bladder excitability via HCN channels on ICCs. Biochem. Biophys. Res. Commun. 485, 535–541. https://doi.org/10.1016/j.bbrc.2017.01.131.

Xiao, J., Nguyen, T.V., Ngui, K., Strijbos, P.J.L.M., Selmer, I.S., Neylon, C.B., Furness, J.B.,

2004. Molecular and functional analysis of hyperpolarisation-activated nucleotide-gated (HCN) channels in the enteric nervous system. Neuroscience 129, 603–614. https://doi.org/10.1016/j.neuroscience.2004.08.027.

Xue, L., Li, Y., Han, X., Yao, L., Yuan, J., Qin, W., Liu, F., Wang, H., 2012. Investigation of hyperpolarization-activated cyclic nucleotide-gated channels in interstitial cells of Cajal of human bladder. Urology 80, 224. https://doi.org/10.1016/j.urology.2012.04.005 e13-224.e18.

Yanagida, H., Inoue, R., Tanaka, M., Ito, Y., 2000. Temperature-sensitive gating of cation current in Guinea pig ileal smooth muscle activated by hyperpolarization. Am. J. Physiol. Cell Physiol. 278, 40–48. https://doi.org/10.1152/ajpcell.2000.278.1.c40.

Yang, S., Xiong, C.jie, Sun, H.mei, Li, X., shuang, Zhang, quan, G., Wu, B., Zhou, D. shan, 2012. The distribution of HCN2-positive cells in the gastrointestinal tract of mice. J. Anat. 221, 303–310. https://doi.org/10.1111/j.1469-7580.2012.01546.x.

Yeh, J., Kim, B.S., Gaines, L., Peresie, J., Page, C., Arroyo, A., 2008. The expression of hyperpolarization activated cyclic nucleotide gated (HCN) channels in the rat ovary are dependent on the type of cell and the reproductive age of the animal: a laboratory investigation. Reprod. Biol. Endocrinol. 6, 2–9. https://doi.org/10.1186/1477-7827-6-35.

Yeh, J., Kim, B.S., Peresie, J., Page, C., 2009. Declines in levels of Hyperpolarization-activated Cation (HCN) channels in the rat ovary after cisplatin exposure. Reprod. Sci. 16, 986–994. https://doi.org/10.1177/1933719109339217.

Young, R.C., 2018. The uterine pacemaker of labor. Best Pract. Res. Clin. Obstet. Gynaecol. 52, 68–87. https://doi.org/10.1016/j.bpobgyn.2018.04.002.

Yue-Chun, L., Guang-Yi, C., Li-Sha, G., Chao, X., Xinqiao, T., Cong, L., Xiao-Ya, D., Xiangjun, Y., 2016. The protective effects of ivabradine in preventing progression from viral myocarditis to dilated cardiomyopathy. Front. Pharmacol. 7, 408. https://doi.org/10.3389/fphar.2016.00408.

Zhang, Y., Zhang, N., Gyulkhandanyan, A.V., Xu, E., Gaisano, H.Y., Wheeler, M.B., Wang, Q., 2008. Presence of functional hyperpolarisation-activated cyclic nucleotide-gated channels in clonal alpha cell lines and rat islet alpha cells. Diabetologia 51, 2290–2298. https://doi.org/10.1007/s00125-008-1166-x.

Zhang, Y., Liu, Y., Qu, J., Hardy, A., Zhang, N., Diao, J., Strijbos, P.J., Tsushima, R., Robinson, R.B., Gaisano, H.Y., Wang, Q., Wheeler, M.B., 2009. Functional characterization of hyperpolarization-activated cyclic nucleotide-gated channels in rat pancreatic β cells. J. Endocrinol. 203, 45–53. https://doi.org/10.1677/JOE-09-0068.