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J Physiol 594.20 (2016) pp 5869–5879

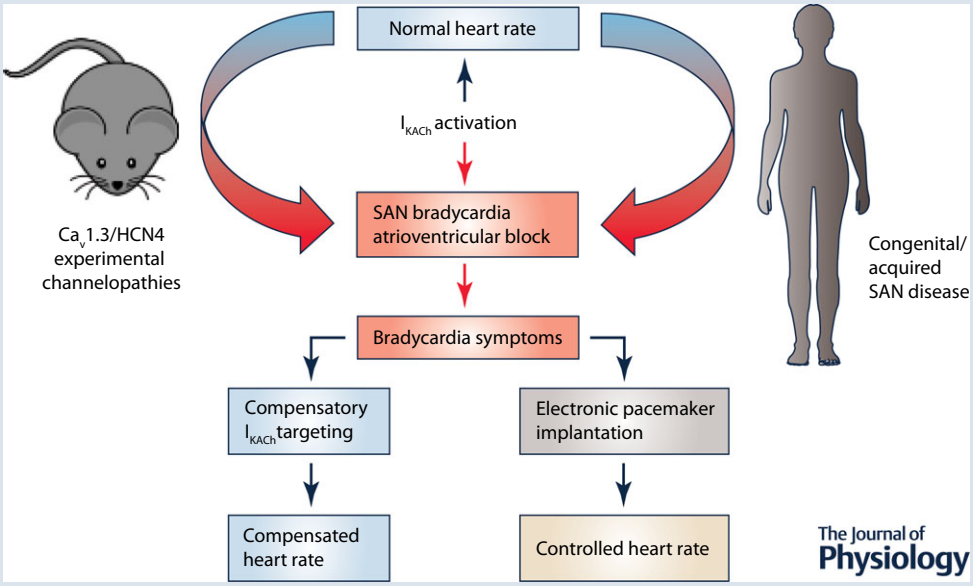
The Journal of Physiology

SYMPOSIUM REVIEW

Rescuing cardiac automaticity in L-type Cav1.3 channelopathies and beyond

Pietro Mesirca[1,2,3], Isabelle Bidaud[1,2,3] and Matteo E. Mangoni[1,2,3]

[1] *Département de Physiologie, Institut de Genomique Fonctionnelle, LabEx ICST, UMR-5203, Centre national de la recherche scientifique, F-34094 Montpellier, France*
[2] *INSERM U1191, F-34094 Montpellier, France*
[3] *Université de Montpellier, F-34094 Montpellier, France*



Abstract Pacemaker activity of the sino-atrial node generates the heart rate. Disease of the sinus node and impairment of atrioventricular conduction induce an excessively low ventricular rate (bradycardia), which cannot meet the needs of the organism. Bradycardia accounts for about half of the total workload of clinical cardiologists. The 'sick sinus' syndrome (SSS) is characterized by sinus bradycardia and periods of intermittent atrial fibrillation. Several genetic or acquired risk factors or pathologies can lead to SSS. Implantation of an electronic pacemaker constitutes the only available therapy for SSS. The incidence of SSS is forecast to double over the next 50 years, with

Matteo Mangoni is Research Director in the Department of Physiology of the Institute of Functional Genomics in Montpellier (France). He obtained his doctoral degree in Physiology at the University of Milan (Italy) with Dario DiFrancesco. His team works on the mechanisms underlying cardiac automaticity using a wide collection of genetically modified mouse strains. **Pietro Mesirca** is Research Associate in the same department. He obtained his doctoral degree in Physics at the University of Bologna (Italy). His work has contributed to the understanding of the antagonistic roles of G protein-activated K^+ (I_{KACh}) and other critical inward currents underlying cardiac automaticity such as I_f and $Ca_v1.3$-mediated $I_{Ca,L}$. **Isabelle Bidaud** is Senior Research Engineer in the same Department. She obtained her doctoral degree in Molecular Biology at the University of Paris VI. She characterized the genetic and pharmacological factors able to regulate heart rate in mice recapitulating disease of cardiac automaticity.

DOI: 10.1113/JP270678

ageing of the general population thus urging the development of complementary or alternative therapeutic strategies. In recent years an increasing number of mutations affecting ion channels involved in sino-atrial automaticity have been reported to underlie inheritable SSS. L-type $Ca_v1.3$ channels play a major role in the generation and regulation of sino-atrial pacemaker activity and atrioventricular conduction. Mutation in the *CACNA1D* gene encoding $Ca_v1.3$ channels induces loss-of-function in channel activity and underlies the sino-atrial node dysfunction and deafness syndrome (SANDD). Mice lacking $Ca_v1.3$ channels ($Ca_v1.3^{-/-}$) fairly recapitulate SSS and constitute a precious model to test new therapeutic approaches to handle this disease. Work in our laboratory shows that targeting G protein-gated K^+ (I_{KACh}) channels effectively rescues SSS of $Ca_v1.3^{-/-}$ mice. This new concept of 'compensatory' ion channel targeting shines new light on the principles underlying the pacemaker mechanism and may open the way to new therapies for SSS.

(Received 3 April 2016; accepted after revision 24 June 2016; first published online 4 July 2016)
Corresponding authors M. E. Mangoni and P. Mesirca: IGF, CNRS UMR-5203, Department of Physiology, 141, rue de la cardonille, F-34094 Montpellier, France. Email: matteo.mangoni@igf.cnrs.fr and pietro.mesirca@igf.cnrs.fr

Abstract figure legend Genetically modified mice carrying loss-of-function in $Ca_v1.3$ or HCN4 channels display bradycardia and heart block (experimental channelopathy). This condition is similar to some forms of inherited sino-atrial node dysfunction in humans. The G protein-activated K^+ current I_{KACh} constantly imposes negative chronotropic and dromotropic effects on the heart rhythmogenic centres under physiological conditions (normal heart rate). However, the action of I_{KACh} becomes detrimental under conditions of diseased heart impulse generation and eventually causes symptoms associated with bradycardia. In humans, these symptoms are managed by implantation of an electronic pacemaker, which constitutes the only currently available therapy. In mice, 'compensatory' genetic or pharmacological targeting of I_{KACh} can rescue bradycardia. This strategy may open the way for new therapeutic options to handle disease of heart automaticity in humans.

Abbreviations AVN, atrioventricular node; $[Ca^{2+}]_i$, intracellular Ca^{2+}; HRV, heart rate variability; RyR, ryanodine receptors; SAN, sino-atrial node; SANDD, sinus node dysfunction and deafness syndrome; SSS, sick sinus syndrome.

Introduction: cardiac pacemaker activity and sinus node dysfunction

Pacemaker activity of the sino-atrial node (SAN) underlies heart automaticity and constantly regulates heart rate. The cardiac conduction system ensures a proper spatial and timely spread of the SAN impulse to the ventricles. SAN automaticity is due to a specialized population of myocytes referred to as 'pacemaker' cells (Mangoni & Nargeot, 2008). These cells are differentiated by the activation of a specialized genetic differentiation programme, which is distinct from that of the working myocardium and display peculiar morphology (Christoffels *et al.* 2010). The electric hallmark of pacemaker cells is the presence of the diastolic depolarization phase of the action potential, which leads the membrane voltage from the maximum diastolic potential to the threshold of the following action potential (DiFrancesco *et al.* 1986). This specific genetic programme of SAN differentiation induces the expression of a set of ion channels that differ qualitatively and quantitatively from those of the other cardiac chambers (Marionneau *et al.* 2005; Chandler *et al.* 2009). In comparison to the myocytes of the working myocardium, pacemaker cells are characterized also by the presence of local intracellular Ca^{2+} (Ca_i^{2+}) release from ryanodine receptors (RyRs) during the diastolic depolarization at discrete points of the subsarcolemmal space (Huser *et al.* 2000; Rigg *et al.* 2000; Bogdanov *et al.* 2001). During spontaneous activity, local Ca_i^{2+} release precedes the cell-wide $[Ca^{2+}]_i$ transient evoked in correspondence to the action potential upstroke phase. The cardiac pacemaker mechanism has been the subject of an intensive research effort during the last 20 years (Mangoni & Nargeot, 2008). Research in the field is centred on three main issues: which specific genetic pathways control pacemaker differentiation; how membrane ion channels and RyR-dependent Ca_i^{2+} release generate spontaneous activity; and the identification of downstream effectors of membrane receptors regulating pacemaker activity. The picture emerging from this intensive and strenuous work is that an intricate and still only partially understood functional association of membrane ion channels and Ca_i^{2+} dynamics generates SAN pacemaker activity (for recent reviews see: Mangoni & Nargeot, 2008; DiFrancesco, 2010; Lakatta *et al.* 2010; Monfredi *et al.* 2013; Capel & Terrar, 2015). Several classes of ion channels driving inward current contribute to the diastolic depolarization, including those for the hyperpolarization-activated current I_f

(DiFrancesco, 2010), L- and T-type voltage-gated Ca^{2+} channels (Mesirca *et al.* 2015), and those for the sustained inward current I_{st} (Mitsuiye *et al.* 2000). In addition, voltage-independent channels and Ca^{2+}-activated conductances have been proposed to contribute to pacemaking. These currents are generally referred to as 'background' conductances. These mechanisms have not been fully understood yet; however, chloride channels and channels belonging to TRP gene families contribute to background currents (see for review Capel & Terrar, 2015). Finally, the Na^+/Ca^{2+} exchanger contributes to pacemaking (Ju & Allen, 1998) via its functional interaction with RyRs (Lakatta *et al.* 2010) and possibly by acting as a 'background' conductance (Kang & Hilgemann, 2004).

Beside the great interest in identifying the molecular pathways involved in pacemaking as a fundamental physiological function, this research field has important translational aspects for clinical cardiology. Diseases of heart automaticity and/or impulse conduction are relatively common conditions. SAN or atrioventricular failures result in low ventricular rate, which is inappropriate to meet the physiological demand (Sanders *et al.* 2014). This condition, commonly referred to as 'bradycardia', is a primary cause of electronic pacemaker implantations. In addition, SAN disease is frequently associated with atrial tachyarrhythmias, including atrial fibrillation and flutter. The 'sick sinus' syndrome (SSS) is a typical example of tachycardia–bradycardia syndromes in which patients alternate from periods of low SAN rate and consequent bradycardia to atrial fibrillation (Semelka *et al.* 2013). The aetiology of SSS disease can be complex. Indeed, a broad spectrum of acquired or genetic causes can induce SAN failure or block of atrioventricular conduction. Among acquired causes, ageing, endocrine dysfunction, inflammation, infection and autoimmune disease, as well as improper medication intake or intoxication, are important risk factors (Semelka *et al.* 2013; Monfredi & Boyett, 2015). Irrespective of the underlying causes, symptomatic SAN disease and conduction block are handled by electronic pacemaker implantation, which constitutes the only currently available therapy. Because of the constantly increasing incidence of SAN disease, the number of electronic pacemaker implantations has been forecast to double over the next 50 years, thus making pathologies of heart automaticity a potential future societal problem (Jensen *et al.* 2014).

Pacemaker activity and models of sinus dysfunction

During the last few years the description of mutations in families showing SAN disease has significantly improved knowledge of the aetiological causes of this disease. These mutations affect a wide range of different gene families, including genes encoding cytoskeletal and structural proteins, membrane ion channels and RyRs (Monfredi & Boyett, 2015). While inherited channelopathies do not account for the majority of SAN diseases, they nevertheless constitute a precious model system to investigate the pacemaker mechanism and to test potential therapeutic strategies. Our group has been the first to introduce the use of mouse SAN pacemaker cells in the field (Mangoni, 2001; Mangoni & Nargeot, 2001), showing the feasibility of this approach and the close similarity between the expression pattern of ionic currents and channels between the mouse SAN and that of other animal models used so far (Marionneau *et al.* 2005). We also showed that this approach was extendable also to atrioventricular node (AVN) (Mangoni *et al.* 2006*b*) and Purkinje fibres (Miquerol *et al.* 2004). These works opened the way to investigate the pacemaker mechanism using genetically modified mice and to associate gene activity to a specific function in automaticity (Mangoni *et al.* 2006*a*). Many laboratories in Europe and North America have since used mouse models with altered pacemaker activity.

While research conducted on transgenic mice undoubtedly shed a new light in the cardiac pacemaker mechanism, it also demonstrated that these models fairly recapitulate SAN and impulse conduction disease observed in humans (see for review Monfredi & Boyett, 2015). This symposium article will focus on models of congenital SAN diseases due to mutations in I_f-mediating hyperpolarization-activated f-channels (HCN) and voltage-gated L-type $Ca_v1.3$ Ca^{2+} channels.

Cardiac f-channels underlie the hyperpolarization-activated current (I_f) in the SAN, as well as in the AVN and Purkinje fibres (DiFrancesco, 2010). The sympathetic and parasympathetic branches of the autonomic nervous system regulate the open probability of f-channels in opposite ways through the intracellular concentration of cAMP (DiFrancesco & Tortora, 1991), which directly binds to the channel C-terminus (Ludwig *et al.* 1998). f-Channels play a major role in SAN pacemaker activity (DiFrancesco, 2006), impulse conduction (Baruscotti *et al.* 2011; Mesirca *et al.* 2014) and heart rate determination in mice and humans (Milanesi *et al.* 2006; Alig *et al.* 2009; D'Souza *et al.* 2014; Baruscotti *et al.* 2016). The relevance of I_f in cardiac pacemaking is underscored by the development of the heart rate reducing drug ivabradine, which is now prescribed in the clinical practice for ischaemic heart disease (Bucchi *et al.* 2007). Importantly, several forms of bradycardia (DiFrancesco, 2015) and tachycardia (Baruscotti *et al.* 2016) induced by mutations affecting the predominant SAN f-channel isoforms HCN4 have been described during the last years.

While I_f is an important inward current, it is not the only ionic current to be activated during the diastolic depolarization phase. L-type $Ca_v1.3$ channels

are also activated during the diastolic depolarization phase of SAN cells (Mangoni *et al.* 2003; Torrente *et al.* 2016). $Ca_v1.3$ channels present peculiar steady-state activation and inactivation properties in comparison to the classic cardiac excitation–contraction coupling L-type $Ca_v1.2$ isoform. In cardiac cells and neurons, $Ca_v1.3$ channel activation and inactivation lie about half way between those of low voltage-activated T-type and high voltage-activated $Ca_v1.2$ L-type channels. Because of these biophysical properties, $Ca_v1.3$ channels play also an important role in regulation of firing in neuro-endocrine cells and in some central neurons (see for recent review Vandael *et al.* 2015) and in the control of dendritic Ca^{2+} oscillations in dopaminergic neurons (Guzman *et al.* 2009). In SAN cells, the $Ca_v1.3$-mediated $I_{Ca,L}$ activation threshold is close to –55 mV under adrenergic activation (Mangoni *et al.* 2003). Even under basal conditions, $Ca_v1.3$-mediated $I_{Ca,L}$ half-activation and -inactivation voltages lie 20 mV negative to that of $Ca_v1.2$-mediated $I_{Ca,L}$, thus distinguishing the function of $Ca_v1.3$ channels as 'pacemaker' channels from that of $Ca_v1.2$ channels contributing to the SAN action potential upstroke (Mangoni *et al.* 2006*a*). Similarly to $Ca_v1.2$, the SAN $Ca_v1.3$-mediated $I_{Ca,L}$ is positively regulated by the cAMP-dependent signalling pathway (Mangoni *et al.* 2003). However, new studies are needed to elucidate the other potential cellular factors that regulate $Ca_v1.3$ channels in SAN cells.

Global gene knockout of $Ca_v1.3$ channels induces a 60–70% reduction in the density of SAN $I_{Ca,L}$ (Mangoni, 2001; Mangoni *et al.* 2003) and a positive shift in the current half-activation voltage (Mangoni, 2001; Zhang *et al.* 2002; Mangoni *et al.* 2003). Consequently, $Ca_v1.3^{-/-}$ mice show bradycardia and atrioventricular block (Platzer *et al.* 2000; Marger *et al.* 2011; Mesirca *et al.* 2016). In addition, $Ca_v1.3$ channels contribute to pacemaking not only by supplying inward current in the full range of voltages of the diastolic depolarization, but also by

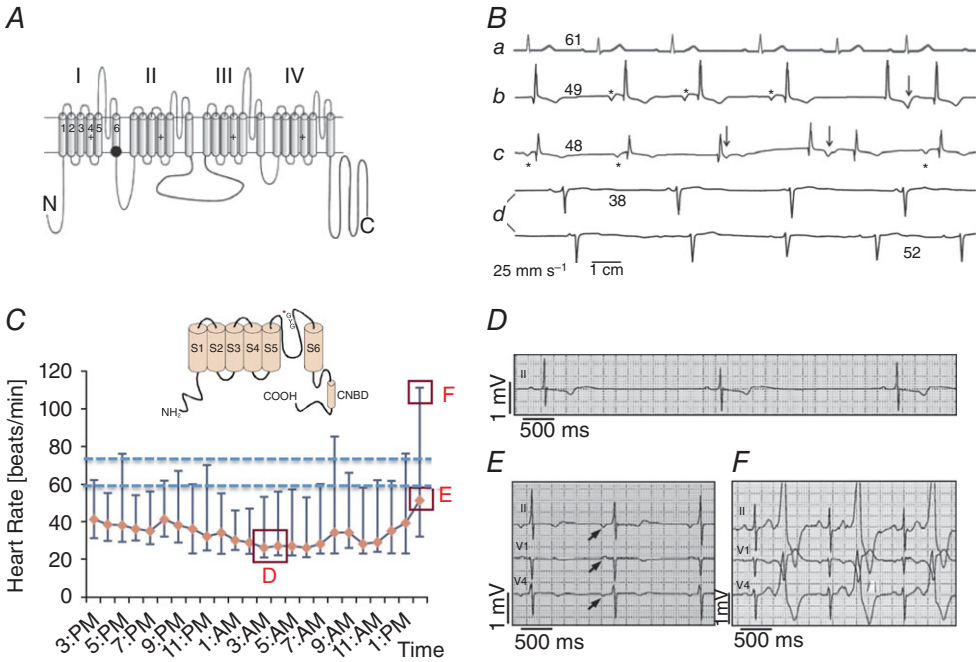


Figure 1. Examples of two congenital dysfunctions of heart automaticity in humans, related to loss-of-function in $Ca_v1.3$ channels (SANDD; *A* and *B*), or familial bradycrdia due to a mutation in the pore sequence of the f-channel HCN4 (*C–F*)

A, schematic transmembrane topology of the α_1 subunit of the $Ca_v1.3$ Ca^{2+} channel. The black dot indicates the position of the SANDD mutation. *B*, sample Holter ECG recordings from individuals with normal heart rate (*a*), or from SANDD affected subjects, who were homozygous for the mutation (*b–d*). The number above each ECG baseline indicates the averaged heart rate. Asterisks mark P waves. Arrows identify ECG time points suggesting P waves coincide with T waves. Adapted from Baig *et al.* (2011), with permission. *C,* daily Holter heart rate profile of a patient with familial SAN dysfunction due to HCN4 pore mutation. Data points indicate hourly averaged data between the maximum and minimum heart rate of a patients carrying the glycine–tyrosine–glycine (GYG) mutation in the pore motif of HCN4. The inset shows a schematic transmembrane topology of the f-channel HCN4 subunit. The asterisk indicates the position of the mutation within the motif of the channel pore. Dashed lines indicate the expected basal heart rate interval for normal subjects. Red boxes mark time points corresponding to ECG samples shown in panels *D–F*. *D*, a sample recording of SAN bradycardia. *E* and *F*, ectopic atrial rhythms and ventricular bigeminisms, respectively. Adapted from Schweizer *et al.* (2014), with permission.

regulating the activity of RyRs (Torrente *et al.* 2016). Previous work has shown that reduction in $Ca_v1.3$-mediated $I_{Ca,L}$ contributes to SAN disease due to loss-of-function of ankyrin-B (Hund & Mohler, 2008). However, only recently it has been possible to show directly that loss-of-function of $Ca_v1.3$ channel gating induces SAN bradycardia and congenital deafness in humans (Baig *et al.* 2011). It has also been proposed that reduction in $Ca_v1.3$-mediated $I_{Ca,L}$ is responsible for bradycardia observed in patients affected by chronic iron overload (Rose *et al.* 2011). Collectively, these works indicate that a significant fraction of SSS and atrioventricular conduction diseases could be ascribed to functional alteration in ion channels contributing to the diastolic depolarization and that mouse models of human channelopathies can provide a valuable tool to test potential molecular and pharmacological rescuing strategies.

The role of the autonomic nervous system input in establishing the hallmarks of sino-atrial and atrioventricular dysfunctions

During the last 10 years, different groups have made an intensive effort to dissect the consequences for pacemaker activity and heart rate following knockout of the predominant SAN f-channel isoform HCN4. Global or cardiac specific knockout of *hcn4* in mice is embryonically lethal (Stieber *et al.* 2003). Conditional *hcn4* knockout in adult mice gave apparently contrasting results, with phenotypes ranging from SAN pauses (Herrmann *et al.* 2007) to SAN bradycardia and quickly developing lethal heart block (Baruscotti *et al.* 2011). The effects on cardiac pacemaker activity of conditional and time-controlled expression of dominant negative HCN4 subunits lacking either cAMP-dependent regulation of the channel gating (Schulze-Bahr *et al.* 2003; Alig *et al.* 2009) or mutated pore sequence silencing channel conductance (Mesirca *et al.* 2014) have been recently described. Incidentally, these mouse lines express mutated HCN4 channels similar to those found in two familial congenital SAN dysfunctions (Schweizer *et al.* 2010; Schweizer *et al.* 2014). In particular, our group showed that genetic silencing of I_f in mice via conditional time- and cardiac-specific expression of non-conducting HCN4 mutant channels (HCN4-AYA) induces complex arrhythmia with SAN dysfunction, atrioventricular block and high incidence of ventricular tachycardia (Mesirca *et al.* 2014). While not being lethal, this phenotype shares qualitative similarities with that reported by Baruscotti *et al.* (2011). This mouse model also recapitulates familial SAN dysfunction induced by a mutation in the same pore motif of the HCN4 channel subunit (Fig. 1), which reduces (but does not eliminate) f-channel conductance (Schweizer *et al.* 2010, 2014). Expression of HCN4-AYA channels induces complete silencing of I_f in the SAN, the AVN and Purkinje fibres. The basal spontaneous activity of automatic cells is slowed, with SAN cells being the most affected cell type in comparison to the other cells of the conduction system.

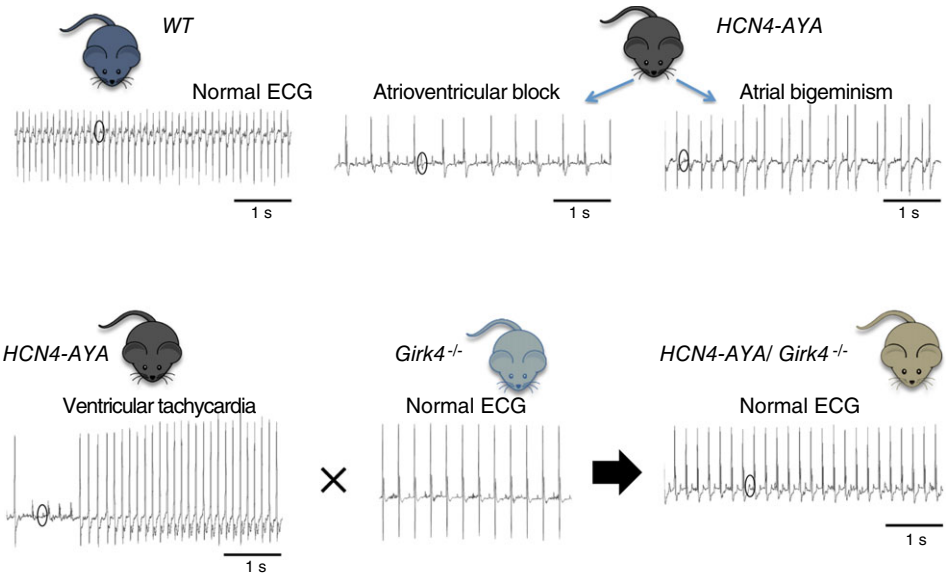


Figure 2. Inactivation of GIRK4 channels rescues the complex cardiac arrhythmia observed in mice with silenced I_f (*HCN4-AYA*)
Wild-type (WT) mice exhibit fast heart rate and a normal ECG profile (top left). In contrast, *HCN4-AYA* double transgenic mice show low SAN rate, 2nd degree atrioventricular blocks and signs of atrial arrhythmia (atrial bigeminism, top centre and right). *HCN4-AYA* mice show also ventricular tachycardia (bottom left). Crossing *HCN4-AYA* mice with *Girk4*$^{-/-}$ mice produces *HCN4-AYA/Girk4*$^{-/-}$ mice with normal ECG (Mesirca *et al.* 2014). Circles indicate P waves in ECG recordings.

Slowing of spontaneous SAN activity by I_f silencing augments the Ca^{2+} content of the sarcoplasmic reticulum, impairs local diastolic Ca_i^{2+} release and delays also the generation of spontaneous $[Ca^{2+}]_i$ transients. This observation suggests that the activity of f-channels is an important factor in coupling membrane voltage to RyR-dependent Ca_i^{2+} release (Lakatta *et al.* 2010). I_f loss-of-function does not affect the relative responsiveness of heart rate to adrenergic activation, and cannot ameliorate SAN dysfunction (SAN pauses) and atrioventricular blocks, nor does it improve ventricular tachycardia (Mesirca *et al.* 2014). However, pharmacological inhibition of the autonomic nervous system by combined injection of atropine and propranolol 'rescues' atrioventricular conduction of HCN4-AYA-expressing hearts. This observation is reminiscent of the effects of the autonomic nervous system in mice conditionally expressing HCN4 channels lacking cAMP-dependent regulation. Indeed, while these mice have lower basal heart rate than their control counterparts, inhibition of the autonomic nervous system abolishes the difference in heart rate of these strains (Alig *et al.* 2009). These observations showed, for the first time, that the autonomic nervous system input in some way regulates the functional role of f-channels in pacemaker activity *in vivo* and that this input is responsible for the manifestations of SAN dysfunction and atrioventricular conduction block. Similarly, pharmacological inhibition of the autonomic nervous system is able to reduce SAN dysfunction and to rescue atrioventricular dysfunction of $Ca_v1.3^{-/-}$ mice, showing that the role of the autonomic nervous system in modulating the symptoms of automaticity failure is not specific to f-channel loss-of-function (Mesirca *et al.* 2016).

The 'compensatory' channel-targeting concept

The G protein-activated K^+ current (I_{KACh}) is involved in the negative chronotropic effect of acetylcholine (ACh) on heart rate (Giles & Tsien, 1975; Giles & Noble, 1976; Noma & Trautwein, 1978; DiFrancesco *et al.* 1989). A gene family comprising four channel subunit isoforms named GIRK1–4 encodes I_{KACh} (Wickman & Clapham, 1995). Only GIRK1 and GIRK4 subunits are expressed in the heart, but mice lacking GIRK4 channels ($Girk4^{-/-}$) are devoid of I_{KACh} since GIRK1 subunits cannot be targeted to the membrane in these mice (Wickman *et al.* 1998). A previous study showed that in rabbit SAN I_{KACh} is activated at concentrations of ACh higher than

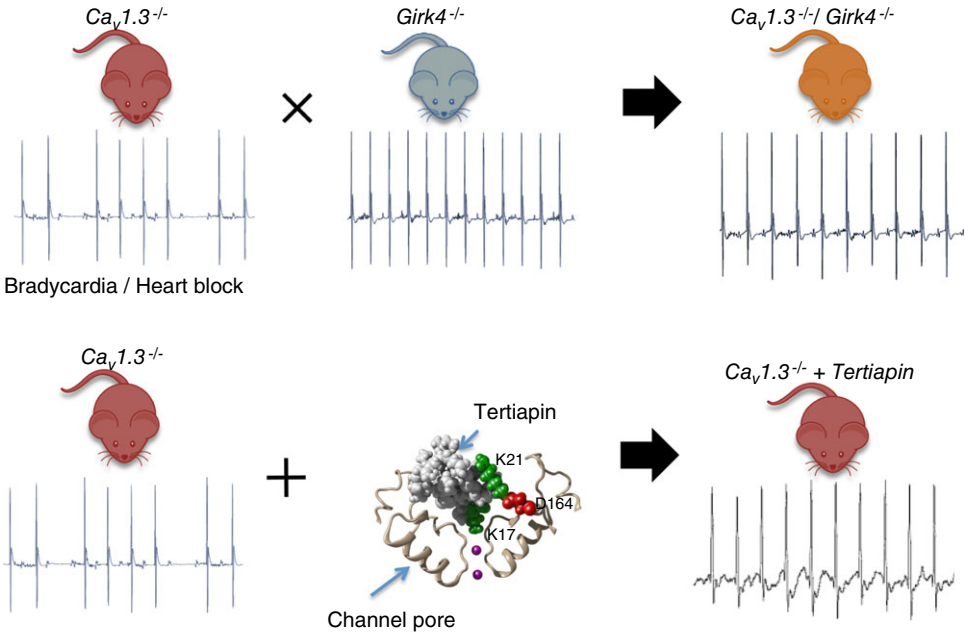


Figure 3. Either genetic ablation (top) or pharmacological targeting (bottom) of Girk4-mediated I_{KACh} channels is able to prevent sick sinus syndrome and atrioventricular block in SANDD mimicking $Ca_v1.3^{-/-}$ mice (Mesirca *et al.* 2016)
Telemetric ECG recordings of $Ca_v1.3^{-/-}$ mice show SAN bradycardia and 2nd degree atrioventricular block (top left). In contrast, $Girk4^{-/-}$ mice present with normal SAN rate and no atrioventricular blocks (top centre). Crossing these mouse lines produces viable $Ca_v1.3^{-/-}/Girk4^{-/-}$ animals with normal heart rate and rhythm (top right). When $Ca_v1.3^{-/-}$ mice undergo an intraperitoneal injection of the GIRK pore blocker tertiapin-Q (tertiapin, bottom centre) normalization of heart rate is observed (bottom right). The central panel shows a close up view of a structural model of tertiapin bound to the GIRK pore. K17 and D164 indicate residues important for tertiapin activity.

those regulating f-channels (DiFrancesco *et al.* 1989). However, I_{KACh} also exhibits a background conductance due to spontaneous channel opening (Ito *et al.* 1994). Inactivation of this background conductance induces a detectable elevation of the basal pacemaker activity of *Girk4*$^{-/-}$ SAN cells (Mesirca *et al.* 2013). In addition, there is solid evidence that I_{KACh} exerts a tonic regulation on heart rate in mice (Wickman *et al.* 1998; Mesirca *et al.* 2013). Indeed, *Girk4*$^{-/-}$ mice show a significant reduction of heart rate variability (HRV) even in the high frequency domain (Wickman *et al.* 1998; Mesirca *et al.* 2013), as well as a delayed recovery of resting heart rate following physical exercise (Mesirca *et al.* 2013). We thus reasoned that abolition of atrioventricular blocks observed in HCN4-AYA mice after administration of atropine and propranolol could be explained by I_{KACh} suppression by atropine, via blockade of muscarinic receptors. We validated this working hypothesis by crossing HCN4-AYA with *Girk4*$^{-/-}$ mice (*HCN4-AYA/Girk4*$^{-/-}$, Fig. 2). These animals are devoid of SAN dysfunction and atrio-ventricular blocks, as well as of the other arrhythmias associated with genetic silencing of f-channels, including atrial and ventricular (Fig. 2) tachycardias (Mesirca *et al.* 2014). In contrast to *Girk4*$^{-/-}$ mice, the recovery of resting heart rate following exercise in *HCN4-AYA/Girk4*$^{-/-}$ mice was similar to that of control animals (Mesirca *et al.* 2014). Taken together, these observations suggested that genetic ablation of GIRK4 (I_{KACh}) channels was restoring equilibrium between ionic currents stimulating (I_f) or braking (I_{KACh}) cardiac pacemaker activity. Inactivation of GIRK4 channels was thus acting as a 'compensatory' mechanism allowing the SAN to control atrial rate and to suppress ventricular tachycardias by normalizing atrio-ventricular conduction.

*Ca$_v$1.3*$^{-/-}$ mice constitute a faithful model of tachy–brady syndromes, since they present with both bradycardia (Platzer *et al.* 2000; Zhang *et al.* 2002; Mesirca *et al.* 2016) and atrial tachyarrhythmia (Zhang *et al.* 2005; Mesirca *et al.* 2016). In addition, the similarity between SSS observed in *Ca$_v$1.3*$^{-/-}$ mice and SAN dysfunction associated with SANDD (Figs 1*A* and 3) indicates that this mouse strain can be relevant for testing new therapeutic approaches. Similarly to *HCN4-AYA* mice, genetic ablation of I_{KACh} in *Ca$_v$1.3*$^{-/-}$ mice produces *Ca$_v$1.3*$^{-/-}$*/Girk4*$^{-/-}$ animals presenting normal heart rate and rhythm (Mesirca *et al.* 2016). Taken together, the phenotype of *HCN4-AYA/Girk4*$^{-/-}$ and *Ca$_v$1.3*$^{-/-}$*/Girk4*$^{-/-}$ demonstrates that it is possible to rescue SAN dysfunction and improve heart rate of genetically modified mice models of congenital channelopathy of heart automaticity. Importantly, pharmacological block of I_{KACh} by the bee venom petide tertiapin mimics rescuing of *Ca$_v$1.3*$^{-/-}$ heart rate by genetic inactivation of GIRK4 channels, which demonstrates that improvement of pacemaker activity is not the result of remodelling phenomena (Fig. 3).

Prevention of atrial tachycardias typical of *Ca$_v$1.3*$^{-/-}$ hearts by inactivation of GIRK4 channels can be due

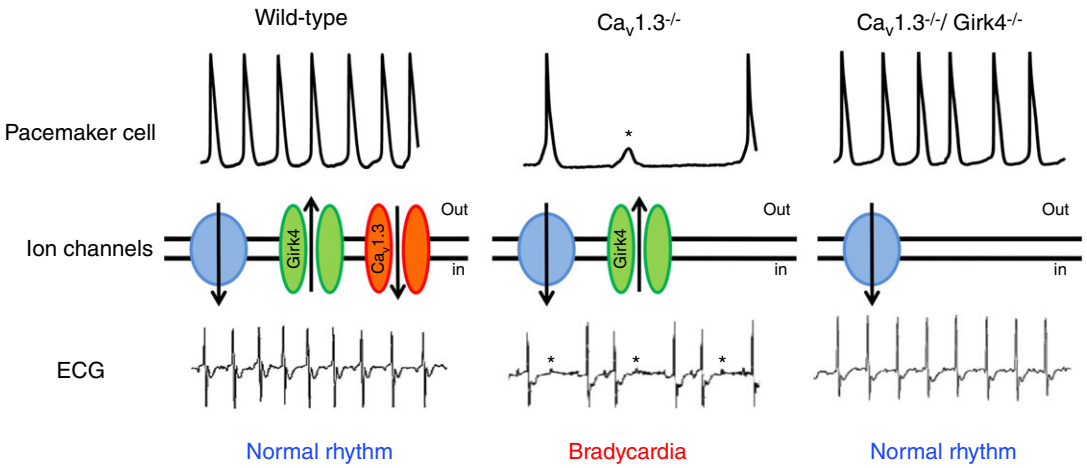


Figure 4. Summary of the principle of re-establishment of the equilibrium among pacemaker mechanisms by 'compensatory' targeting of an ion channel in the mouse heart
Top, samples of consecutive spontaneous action potentials recorded in isolated SAN pacemaker cells from wild-type (left), *Ca$_v$1.3*$^{-/-}$ (centre) and *Ca$_v$1.3*$^{-/-}$*/Girk4*$^{-/-}$ (right) mice. Middle, ion channels in the plasmalemma of SAN pacemaker cells. Green identifies I_{KACh} (GIRK4) channels, orange shows L-type Ca$_v$1.3 channels and the blue circle represents the idealized sum of other inward currents involved in the generation of pacemaker activity. Bottom, samples of ECGs of wild-type mice with normal heart rate (left), *Ca$_v$1.3*$^{-/-}$ mice showing slow SAN rate and atrioventricular blocks leading to bradycardia (centre), or *Ca$_v$1.3*$^{-/-}$*/Girk4*$^{-/-}$ mice with 'compensated' heart rate (left). Asterisks indicate failure of action potential generation (top line) or atrioventricular blocks (bottom line). Adapted after permission from Mesirca *et al.* (2016).

to normalization of the atrial action potential duration and/or to improvement of SAN rate and rhythm (Mesirca *et al.* 2016). Investigation of the mechanism underlying rescuing of heart automaticity and impulse conduction in $Ca_v1.3^{-/-}$ mice by I_{KACh} ablation shows that targeting GIRK4 channels reduces the cholinergic negative chronotropic response in rhythmogenic centres. Since cholinergic I_{KACh} activation generates an outward current it can be expected that loss of f- or $Ca_v1.3$ channels will reduce the depolarization reserve of SAN pacemaker cells. In this context, analysis of the net current flowing during the SAN diastolic depolarization indicates that in $Ca_v1.3^{-/-}$ pacemaker cells ACh switches the net diastolic current from inward to outward, predicting arrest of automaticity. In contrast, the net diastolic current is maintained in the inward direction in $Ca_v1.3^{-/-}/Girk4^{-/-}$ SAN cells even under ACh, thus predicting regular pacemaking (Mesirca *et al.* 2016).

The concept of 'compensatory' ion channel targeting is summarized and idealized in Fig. 4. Here the behaviour of spontaneous activity is compared with heart rate and rhythm of wild-type mice, as well as with mice having $Ca_v1.3$ loss-of-function and with $Ca_v1.3^{-/-}/Girk4^{-/-}$ mice showing 'compensated' pacemaker activity and heart rate. SAN automaticity depends on the equilibrium between inward and outward ionic currents, which regulate pacemaker activity in opposite ways. Normal SAN rate and atrioventricular conduction are possible when inward and outward currents balance in such a way that the direction of the sum of membrane currents during the diastolic depolarization is inward. Under this condition, the SAN will generate constant pacemaker activity at a rate determined by the inputs of the sympathetic and parasympathetic branches of the autonomic nervous system. Similarly the AVN will conduct the SAN impulse to the ventricles in a 1:1 ratio because the depolarization reserve of conduction cells will allow reaching the action potential threshold. This balance is impaired following $Ca_v1.3$ loss-of-function, and during opening of I_{KACh} channels the net current will switch in the outward direction. This will eventually cause failure in the generation of SAN (or AVN) action potentials (asterisk in Fig. 4) and consequent low SAN rate with pauses and atrioventricular blocks (Mesirca *et al.* 2016). Targeting I_{KACh} channels in $Ca_v1.3$-deficient cells will restore the balance between inward and outward currents preventing SAN bradycardia and SAN pauses, and allowing constant 1:1 conduction through AVN. While Fig. 4 shows an example of $Ca_v1.3$ loss-of-function, results indicate that the concept could be extended to loss or decreased function of other channels involved in pacemaking. Indeed, as discussed above, genetic ablation of I_{KACh} in *HCN4-AYA* mice effectively rescues the complex arrhythmic profile observed in these mice (Mesirca *et al.* 2014). This additional evidence is suggestive of a high degree of functional redundancy between inward ionic currents contributing in the pacemaker mechanisms, so that the loss of one current can be compensated by ablation of I_{KACh}. Interestingly, I_{KACh} ablation seems able to compensate also for partial loss of RyR-dependent Ca^{2+} release in SAN cells, provided that $Ca_v1.3$ channels are still present (Mesirca *et al.* 2016).

Conclusions

Failure in the generation or regulation of the cardiac impulse is a common clinical condition which, with the ageing of the population, is becoming a societal issue. The aetiological and mechanistic bases underlying congenital diseases in SAN automaticity and atrioventricular conduction have remained undefined for many years. However, the discovery of mutations in ion channels involved in pacemaker activity sheds new light on these pathologies, showing that some of them were indeed channelopathies (Dobrzynski *et al.* 2007; Monfredi & Boyett, 2015).

The development and functional exploration of mouse lines that recapitulate SSS and associated arrhythmias allows new therapeutic strategies to be tested with the aim of offering alternative or complementary therapies to the implantation of electronic pacemakers. 'Compensatory' I_{KACh} targeting will possibly constitute a new research avenue to manage bradycardia and SSS (Mesirca *et al.* 2016). A corollary consideration emerging from this work is that, at least in the case of channelopathies of cardiac pacemaker activity, while I_{KACh} constitutes a physiological mechanism regulating normal heart rate under physiological conditions, its activity becomes highly detrimental to impulse generation and conduction under SSS conditions. This unwanted action eventually causes the symptoms of SSS and associated arrhythmias in mice. Thus, compensatory I_{KACh} targeting aims chiefly to re-establishment an intrinsic equilibrium in pacemaker mechanism that is lost during the onset and progression of SAN disease. Future works will define the clinical applicability of this approach and whether, beside I_{KACh}, other ion channels could be targeted to improve and normalize heart rate in cardiac disease.

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Additional information

Competing interests

The authors declare no conflicts of interest.

Author contributions

All authors have contributed to writing the paper. All authors approved the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors, qualify for authorship, and all those who qualify for authorship are listed.

Funding

The authors' work is supported by the Agence Nationale pour la Recherche (ANR, Paris) grant ANR-15-CE14-0004. P.M was supported by the CavNet a Research Training Network (RTN) funded through the European Union Research Programme (6FP) MRTN-CT-2006-035367. The group is a member of the Laboratory of Excellence 'Ion Channel Science and Therapeutics' (ICST) supported by a grant from ANR (ANR-11-LABX-0015).

Acknowledgements

We thank Prof. Philippe Chevalier (CHU, Lyon, France) for helpful discussion on SSS. We are indebted to the staff of the RAM animal facility of Montpellier for managing mouse lines.