

# Channels

## Potassium channels in the sinoatrial node and their role in heart rate control

--Manuscript Draft--

<b>Manuscript Number:</b>	KCHL-2018-0029R1
<b>Article Type:</b>	Review
<b>Keywords:</b>	Sinoatrial node; potassium channels; heart rate; pacemaking; ATP sensitive potassium channels
<b>Corresponding Author:</b>	Andrew Tinker William Harvey Research Institute London, UNITED KINGDOM
<b>First Author:</b>	Qadeer Aziz
<b>Order of Authors:</b>	Qadeer Aziz Yiwen Li Andrew Tinker
<b>Manuscript Region of Origin:</b>	UNITED KINGDOM
<b>Abstract:</b>	Potassium currents determine the resting membrane potential and govern repolarisation in cardiac myocytes. Here, we review the various currents in the sinoatrial node focussing on their molecular and cellular properties and their role in pacemaking and heart rate control. We also describe how our recent finding of novel a ATP-sensitive potassium channel population in these cells fits into this picture .

Ref.: Ms. No. KCHL-2018-0029 Potassium channels in the sinoatrial node and their role in heart rate control Channels

We thank the Editor-in-Chief for their kind comments and the opportunity to revise our review.

### **Reply to reviewer**

1. The reviewer writes “This review provides an overview of SAN electrophysiology and then in more detail the roles of KIR6x channels in repolarization and pacemaking activity. The review is quite general until about page 9 of 12 when it gets into KATP channels, when the expertise of the authors comes more to the forefront. The first part of the review, while correct, could certainly benefit from a more detailed literature review with a bit more background and critical analysis, although the authors do note the important remaining controversies. The second part of the review deals with some current insight provided by the authors on the roles of Kir6.1 in SAN activity and cardiac conduction, and KATP channels in hypoxia.”

Reply:- We have included more detail on

- a. The nature of the pacemaker in particular recent literature on the calcium clock.
- b. Cyclic AMP and PKA signalling in the SA node.
- c. Pathological changes in the SA node.

The new text is in red and there are an additional twenty-six references.

2. The reviewer writes “Minor: FIG.1. A scale showing the 0 mV position and the MDP would be helpful here. & Page 12. By 'the' SAN artery.”

Reply:- We have made the changes as requested and given the MDP in the legend.

# **Potassium channels in the sinoatrial node and their role in heart rate control**

Qadeer Aziz, Yiwen Li and Andrew Tinker\*

William Harvey Heart Centre,  
Barts & The London School of Medicine & Dentistry,  
Queen Mary, University of London  
Charterhouse Square,  
London, EC1M 6BQ

Tel: 020 7882 5783, E-mail: a.tinker@qmul.ac.uk

\* Corresponding Author

Keywords: Potassium channel, ATP-sensitive potassium channel, heart rate, pacemaking, sinoatrial node.

## **Abstract**

Potassium currents determine the resting membrane potential and govern repolarisation in cardiac myocytes. Here, we review the various currents in the sinoatrial node focussing on their molecular and cellular properties and their role in pacemaking and heart rate control. We also describe how our recent finding of a novel ATP-sensitive potassium channel population in these cells fits into this picture .

## **Introduction**

The regular beating of the heart is generated by intrinsic pacemaker activity in the sinoatrial node (SAN). Heart rate can be easily measured and is known to be associated with disease in a number of epidemiological studies. For example, in Paris Prospective Study I the risk of sudden death increased over three fold (relative risk 3.9) in those with a resting heart rate >75 beats per minute compared to those with one under 60 (1). Furthermore, the increase in heart rate during exercise and the rate of recovery of rate after cessation of exercise were also strongly associated with sudden death (1). Moreover, direct intervention to lower heart rate using drugs with predominant effects on the SAN can improve outcomes in heart failure (2).

The pacemaker action potential has a characteristic morphology with a diastolic depolarisation before a threshold is reached with a consequent action potential. Figure 1 shows this in single mouse SAN cells studied in the current clamp mode of the patch clamp. Exactly how the intrinsic pacemaker clock is generated is controversial. Historically the rhythmical oscillations of membrane potential were thought to be governed by the hyperpolarisation-activated cyclic nucleotide-gated cation channel (also known as the “funny” current,  $I_f$ ) constituted of the product of the HCN genes (largely HCN4) (3). However, published data now show the importance of intracellular calcium cycling

specifically diastolic calcium release from the sarcoplasmic reticulum generating an inward current via the sodium-calcium exchanger (4). It is likely that these two mechanisms are in fact highly interdependent. Furthermore, this oscillatory electrical activity is subject to regulation by the autonomic nervous system and that allows heart rate to increase flexibly with increased demand such as during exercise. In this review, we consider the role of potassium channels in shaping SAN electrophysiology and their part in heart rate control. We also discuss our recent work on a new player in SAN excitability namely ATP-sensitive potassium channels ( $K_{ATP}$ ) constituted of Kir6.1 subunits.

Figure 1 here.

### **An overview of the electrophysiology of the SAN**

The SAN sits in the upper part of the right atrium laterally to the entrance of the superior vena cava. Textbooks show a characteristic pacemaker action potential, however the SAN is actually quite a heterogeneous structure, both morphologically and electrophysiologically (5). The primary pacemaker can also shift location with changes in autonomic tone (6). With this caveat in mind it is helpful to consider the main currents underlying excitability.

The rapid initial depolarisation of the action potential, also known as phase 0, is not as fast as in ventricular tissue and is mediated largely by L-type calcium currents. At the molecular level SAN cells express both  $Ca_v1.2$  and  $Ca_v1.3$ .  $Ca_v1.2$  is the classic L-type channel present throughout the heart that is responsible for calcium entry promoting calcium-induced calcium release from the sarcoplasmic reticulum and in the SA node is responsible for some of the action potential depolarisation (7). In contrast,  $Ca_v1.3$  activates at more hyperpolarised potentials and contributes to pacemaking (8,9). Sodium currents are less

obvious as the main driver of depolarisation. However, tetrodotoxin-resistant and sensitive currents have been described and these may be present in the more peripheral parts of the node adjacent to the atrial tissue (5). Sodium channel mutations in SCN5A result in cardiac conduction disease (10). SCN10A has also been associated with conduction albeit in the atrioventricular node (11). Repolarisation of the action potential is achieved by inactivation of L-type calcium currents and opening of a number of potassium channels. The latter are discussed in more detail below. The SAN action potential lacks a notch and plateau phase (phase 1 and 2) that is characteristic of the ventricular action potential.

The second feature of SAN electrophysiology is the slow diastolic depolarisation also known as the pacemaker potential. In the initial phase this is mediated by sodium influx via  $I_f$ , largely constituted by the HCN4 subunit, and as depolarisation occurs progressively by T-type calcium channels and finally L-type calcium channels, specifically  $Ca_v1.3$ , just prior to threshold and initiation of phase 0.  $Ca_v1.3$  knockout mice have highly unstable pacemaker function with bradycardia and episodes of sinus pauses (12). The major T-type subunit in the adult animal is  $Ca_v3.1$  and knockout mice are bradycardic (13). This slow diastolic depolarisation is opposed by inward rectifier potassium currents and other background currents (see below). Figure 2 summarises the important currents underlying the SAN action potential.

Figure 2 here.

### **How is the rhythmicity of the diastolic depolarisation generated?**

One of the major recent advances has been an appreciation of the importance of intracellular calcium cycling in the genesis of the rhythmicity of the diastolic depolarisation (4,14). In addition to the membrane ion channels detailed above there is also expression of

the sodium-calcium exchanger in the sarcolemma of the SAN myocytes (15,16). The sodium-calcium exchanger transports three sodium ions for one calcium and thus has the potential to generate a net inward current when extruding calcium (17). Calcium is released from the sarcoplasmic reticulum even during diastole when the cell is quiescent and in ventricular cells this is manifest as calcium sparks (18). In SAN myocytes however these local calcium releases are larger involving a number of release sites (19-21). In permeabilised cells the effects of membrane ion channels are removed and in these conditions these events are periodic in contrast to the stochastic nature of sparks in ventricular myocytes (22). The SA node does not have t-tubules and calcium release is sub-sarcolemmal. The timing of the spontaneous calcium release is in the latter part of the diastolic depolarisation and the activation of the inward sodium-calcium exchanger current is exponential ultimately leading to the threshold potential (15,21). The SAN seems to have higher levels of the calcium pump, SERCA2, present in the membranes of the sarcoplasmic reticulum and in inducible knockout mice the heart rate is substantially slowed (23,24). In addition, phospholamban, which in its unphosphorylated state inhibits SERCA2, is reduced in expression and basally phosphorylated compared to the ventricle (25). Pacemaking and spontaneous calcium release are inhibited by cyclopenzoic acid and ryanodine and heart rate is slowed in a mouse with knockout of the sodium-calcium exchanger (16,19,20,26). These observations all support the importance of a fundamental calcium clock in generating the rhythmic oscillator SAN discharge which integrates with the activity of membrane ion channels.

### **Potassium channels and pacemaking**

Given these features of SAN electrophysiology, the opening of potassium channels can contribute in two ways. In the first action potential repolarisation can be accelerated attenuating the contribution of the action potential to the cycle length and thus potentially

increasing heart rate. In contrast an increase in potassium conductance during the slow diastolic depolarisation will increase the maximum diastolic potential and slow the rate of pacemaker depolarisation leading to a slower heart rate. The exact net contribution depends on the properties of the potassium current. If the current predominates around the resting membrane potential, such as with inwardly-rectifying potassium channels, then the net effect will be on the diastolic depolarisation. In contrast if active at more depolarised potentials this could predominantly influence repolarisation. In principle both effects might occur and the net effect on heart rate would be difficult to predict. Modelling in these circumstances might be helpful in aiding the understanding of the physiological consequences of changes in a particular potassium conductance. The regulation of these currents could also be potentially important: increased potassium currents during the diastolic potential leading to heart rate slowing. It is also important that action potential duration adapts by shortening during high heart rates otherwise regular pacemaking would fail. Adrenergic modulation of repolarising potassium conductances is a mechanism to ensure that and this is discussed below.

### **G-protein gated inwardly rectifying potassium (GIRK) currents and other background currents**

All cardiac myocytes express a strong inwardly-rectifying potassium current. In ventricular cells this is known as  $I_{K1}$  and is accounted for by members of the Kir2.0 family of inward rectifiers (9,27,28). In contrast in the SAN and atrial cells the current is less strongly inwardly-rectifying but is characteristically increased by muscarinic agonists such as acetylcholine and carbachol (" $I_{KACH}$ ") (29). This current is a heteromultimer of Kir3.0 subunits that in cardiac cells is constituted of Kir3.1 and Kir3.4 with perhaps some homomultimers of Kir3.4 (30-32). The differential expression is not absolute for example there are some data indicating expression of  $I_{KACH}$  in the ventricle (33).



Activation of  $I_{KACH}$  is characteristically inhibited by muscarinic antagonists and activation is inhibited by pertussis toxin implicating heterotrimeric G-proteins in the regulation (34). After some controversy, it became clear that it was the  $G_{\beta\gamma}$  subunit, not the inhibitory  $G_{\alpha}$ , that directly activated the channel complex and in many ways this is now the paradigmatic example of modulation of an effector by  $G_{\beta\gamma}$  (35,36). The domains on the Kir3.0 subunits that bind the  $G_{\beta\gamma}$  subunits and also the key residues on  $G_{\beta\gamma}$  have been mapped (36,37). There are crystal structures of the channel complex with and without  $G_{\beta\gamma}$  subunits bound (38). Anionic phospholipids particularly phosphatidylinositol-(4,5)-bisphosphate and sodium are known to be key modulators of the gating and a number of site-directed mutagenesis studies together with structural work have suggested models predicting how this might occur (38-40). However the inhibitory G-protein  $\alpha$  subunit also participates in determining the selectivity of activation but not directly in activation itself (41). There are strands of evidence pointing to models in which the inhibitory heterotrimeric G-protein is complexed with the channel and receptor prior to activation (42-46).

The pathway delineated by genetic, physiological and pharmacological studies involved the activation of  $M_2$  muscarinic receptors and the dissociation of the inhibitory heterotrimer with the  $G_{\beta\gamma}$  activating GIRK channels in the SAN (47-50). However the GTP bound inhibitory  $G_{\alpha}$  subunit can also inhibit adenylate cyclase and reduce levels of cAMP reducing  $I_f$  (51). Thus there are two possible mechanisms for the reduction of heart rate via the parasympathetic arm of the autonomic nervous system: one involving GIRK and another via  $I_f$ . The relative importance of these has been debated over the years. Initially, low receptor occupancy was associated with  $I_f$  inhibition whilst higher receptor occupancy was needed for  $I_{KACH}$  activation (52). The cloning of the Kir3.0 channel subunits enabled the development of mice with global genetic deletion of GIRK4 (*knj5*). These mice together with other experimental observations clearly implicate GIRK channels and  $I_{KACH}$  in heart rate regulation

in physiological conditions (48,53).

GIRK channels are also important in the recovery of heart rate: GIRK4 knockout mice when exercised show a delay in the time taken to return to the resting heart rate (54). This is intriguing as a delayed recovery of heart rate with exercise is associated with poor outcomes (55). Regulator of G-protein signalling 6 (RGS6) is also critical to heart rate regulation (56,57) and acts as the predominant GTPase activating protein for inhibitory G-proteins in the SAN. The net result of this is to inhibit signalling but it also delays the recovery to the inactive state once the agonist is withdrawn. Intriguingly, in our recent genome wide association study in man examining the genetic architecture of heart rate response to exercise we implicated the genes encoding M<sub>2</sub> muscarinic receptor, acetylcholinesterase and RGS6 in determining recovery responses after exercise (58). Furthermore, genetic deletion or pharmacological inhibition of GIRK4 may also ameliorate the bradycardic and proarrhythmic effects of HCN4 and Ca<sub>v</sub>1.3 deletion (59,60).

### **Repolarising currents**

The major repolarising currents in ventricular myocytes are I<sub>Kr</sub> and I<sub>Ks</sub> and both components are also present in SAN myocytes (9,61). I<sub>Kr</sub> is named as such because it is rapidly activating and the currents are characteristically inwardly rectifying due to its unique inactivation properties (62). I<sub>Kr</sub> is composed of hERG (human ether-a-go-go related gene) and possibly a β subunit of the KCNE family though this is still controversial (63,64). I<sub>Kr</sub> is an important current in SAN cells (65,66). For example, the inhibitor E-4031 leads to significant perturbation of the SAN action potential and dofetilide (another blocker) slows pacemaking (61). In contrast, I<sub>Ks</sub> is characteristically activates slowly and the channel complex is formed by KCNQ1 (Kv7.1) and the β subunit KCNE1 (67,68). There may be some species differences in the relative magnitudes of I<sub>Kr</sub> and I<sub>Ks</sub> but I<sub>Ks</sub> is also clearly present in the SAN

(69,70). One key property of these currents, in particular  $I_{Ks}$ , is the augmentation by  $\beta$  adrenergic signalling: it is key in shortening the action potential duration during high heart rates (70,71). Activation of  $\beta$  receptors leads to PKA stimulation and direct phosphorylation of residues S27 and S92 in the channel N-terminus (72). The increase in current requires KCNQ1 to be associated with KCNE1 and is dependent on a protein kinase A anchoring protein (AKAP) *AKAP9* (72,73). Unlike  $I_{Ks}$  regulation, there is no agreement on whether  $I_{Kr}$  currents can be increased by  $\beta$  adrenoreceptor modulation (74,75). Uniquely in the SAN, calcium-calmodulin kinase II may activate  $I_{Ks}$  and intriguingly this could affect calcium handling and channel modulation too (76).

Background potassium currents carried by twin pore channels are difficult to investigate given their poor pharmacology. However a recent study using cardiac-specific TREK1 knockout mice revealed that these mice were bradycardic and predisposed to sinus arrest (77). Thus it seems likely that TREK1 also contributes to repolarisation.

### **$K_{ATP}$ channels underlie repolarising currents in the SA node**

ATP-sensitive potassium channels are widely distributed in the heart. In general the focus has largely been on channels present in the ventricle but characteristic currents can also be recorded in the atria and in the conduction system including the SAN (78,79). The defining property of these channels is their sensitivity to intracellular nucleotides and they are activated by declining ATP levels and/or increasing magnesium ADP levels. The channel complex is made up of four pore-forming inwardly-rectifying Kir6.0 subunits (Kir6.1, Kir6.2) together with four regulatory sulphonylurea receptors (SUR1, SUR2A, SUR2B) (80-82). We now understand these channels well as molecular machines underpinned by exhaustive mutagenesis work and more recently by structural studies. Cryo-electron microscopy has shown how ATP binds to the pore-forming subunit and how MgADP

interacts with the nucleotide binding domains in the sulphonylurea receptor (83-85). Despite the initial description of these channels in the heart (86) there are still questions as to their exact physiological role. Kir6.2 is thought to underlie the classic current present in pancreatic  $\beta$  cells and ventricular cardiomyocytes (87). Kir6.2 global knockout mice are unable to tolerate high intensity exercise partly due to impaired cardiac performance (88).

There has been comparatively less work on the Kir6.1 subunit though it has been known since it was first cloned that it is widely expressed (89). Furthermore, there exists, particularly in smooth muscle, a  $K_{ATP}$  channel with a lower single-channel conductance (35 pS vs 70 pS) with an absolute dependence on cellular nucleotide diphosphates for channel opening. In some papers this led to the channel being called a “ $K_{NDP}$ ” (90). In addition, ATP seems less potent in causing channel inhibition (90,91). This current is recapitulated in heterologous expression systems by the co-expression of Kir6.1 and SUR2B (92) and smooth selective deletion of Kir6.1 (*kcnj8*) in mice abolished the current present in isolated single smooth muscle cells (93).

$K_{ATP}$  currents are present in single isolated SAN cells and they were abolished in cells isolated from the Kir6.2 knockout mouse (94). Interestingly single channel studies revealed a single channel conductance of  $\sim 50$  pS in contrast to the situation in the ventricle where it is closer to 70-80 pS (95). There are reasons to believe that pacemaker function may also be influenced by  $K_{ATP}$  channels containing the Kir6.1 subunit. Mice with global genetic deletion of Kir6.1 develop SAN failure and also heart block which leads to sudden death (93,96). In our own studies we have studied mice with selective deletion of Kir6.1 in vascular smooth muscle or endothelium (93,97). These mice do not show the rhythm disturbance even with provocations that might provoke vasospasm and suggest by exclusion a potential role for Kir6.1 in the SA and atrioventricular node (93,97).

We have explored this question in the mouse using *cre/loxP* technology (98). We

made use of a cre driver line that allows selective deletion of genes within the adult conduction system on the addition of tamoxifen (98,99). The resulting knockout mice are labelled as cKO. We first isolated  $K_{ATP}$  currents in single SAN cells and find that only approximately half the cells contain currents (98). This is not surprising given the heterogeneous electrophysiological nature of the SAN (100). Importantly this number of cells was significantly reduced in cKO mice (98). The pharmacology of the response is also interesting with diazoxide activating and tolbutamide inhibiting and this profile is more typical of SUR1 than SUR2 (101). In murine atrial myocytes SUR1 underpins a significant population of  $K_{ATP}$  channels (102). Furthermore when action potentials were measured in single SAN cells, there was delayed repolarisation in cKO cells resulting in prolonged cycle length (98).

The murine model also allows the investigation of the *in-vivo* physiological consequences of the Kir6.1 deletion (103). Using implanted telemetry probes, we measured heart rate and ECG parameters in awake mice over a prolonged period. cKO mice are bradycardic and in some mice there were episodes of sinus arrest which were not seen in littermate controls (98). Furthermore, there was an indication of atrioventricular nodal dysfunction with an increase of the PR interval on the ECG (98). We also saw some modest pathological changes with fibrosis in the SA node in a few of the cKO mice (98). The phenotype is not as pronounced as in the global Kir6.1 knockout animal but the impairment of vascular reactivity and lack of protective responses may accelerate pathological damage (93,98).

In summary, our recent studies show that  $K_{ATP}$  channels are constitutively active in SA nodal cells. The current seems to influence repolarisation predominantly and this results in an increased cycle length. In-vivo this leads to bradycardia but there was also evidence of sinus pauses, heart block and pathological changes in the SA node (98). It is surprising that

there are no effects on the maximum diastolic membrane potential. Kir6.1 is a member of the inwardly rectifying family of potassium channel however the currents are more outwardly rectifying (98). Additionally, it is plausible that there may be some cyclical regulation of  $K_{ATP}$  channel activity during repolarisation perhaps by calcium and/or cAMP and protein kinase A to explain this paradox. Signalling via cAMP seems to be significantly adapted in the SA node. Adenylyl cyclase is constitutively active and this leads to basal protein kinase A activation (104). The SA node in contrast to the ventricle expresses calcium sensitive adenylyl cyclase isoforms (105). PKA activity, and also that of calcium calmodulin dependent kinase II, is necessary for normal pacemaking as inhibitors of these kinases lead to significant slowing of beating (25,106). Given the sensitivity of both adenylyl cyclase and calcium calmodulin dependent protein kinase II to calcium there may also be cyclical variations in activity that, in addition to phosphorylating phospholamban, may also phosphorylate  $K_{ATP}$  channels. On the background of high phosphatase activity this may lead to variations in beat-to-beat channel activity and account for the prominence of currents during repolarisation. It is also known that  $K_{ATP}$  channels can be regulated by calcineurin (107,108). It is known that Kir6.1-containing channels are prominently regulated by hormonal pathways and protein kinases both in heterologous and native tissues (93,109,110).

### **The response of the SAN to hypoxia**

The sinus node is prone to disease in particular sick sinus syndrome which leads to a pathologically slow heart rate and can be accompanied by sinus arrest leading to dizziness and loss of consciousness (111,112). The disease increases in incidence with age and has been attributed to progressive cell loss and fibrosis (111,112). In addition, atrial fibrillation can be accompanied by sinus node disease and “tachy-brady” syndromes are well described (113). However it also clear that there are changes in the expression of relevant pacemaking genes

in the SA node with age. For example HCN4 and SERCA2 decrease in expression and this may be responsible for the decline in intrinsic heart rate with age (14,113-116). It may also be contributing to physiological and pathological adaptations too (117,118). The SAN is supplied by SAN artery which usually arises from the right coronary artery in man though there are anatomical variations. The SA node responds to hypoxia and ischaemia with heart rate slowing and ultimately failure due to exit block from the SA node into the atrium (119). In modelling work, a major component of the response to hypoxia is the opening of  $K_{ATP}$  channels (119). Potential shortening of the action potential duration is offset by increases in calcium current but the membrane potential becomes hyperpolarised and the pacemaker depolarisation is slowed. This results in bradycardia and ultimately SAN failure. Interestingly, these effects are exacerbated by concomitant increased vagal activity (119).

We investigated this issue in a different way (98). Using an *ex-vivo* Langendorff preparation we measured spontaneous firing using a multielectrode array. We measured the sinus node recovery time by overdrive pacing the endogenous activity and then stopping the pacing and measured the time it takes for the SAN to recover spontaneous activity. This measure is used clinically to assess SAN function and in hypoxic conditions in control mice we found it became prolonged (98). This may be a protective response where a reduction in activity helps preserve the viability of SAN myocytes. In contrast in the cKO mice the SAN recovery time did not prolong with hypoxia and under resting conditions was longer. This lack of flexibility may promote SAN damage. The contribution of  $K_{ATP}$  to SAN physiology and pathophysiology is summarised in Figure 3.

[Figure 3 here.](#)

## **Summary**

Potassium channels play key roles in determining SAN repolarisation and the behaviour of the pacemaker potential. In this review we have placed in context the contribution of  $K_{ATP}$  currents to heart rate regulation revealed in our recent studies.



**Sources of funding:** This research was supported by the British Heart Foundation (RG/15/15/31742) and was facilitated by the NIHR Biomedical Research Centre at Barts.

**Disclosures:** None and the authors have no conflicts of interest.

## Figure Legends

**Figure 1.** The figure shows a recording of spontaneous action potentials in a single isolated SAN myocyte recorded in current clamp in the whole cell configuration of the patch clamp. It illustrates the characteristic morphology of the action potential. The line indicates 0 millivolt and the maximum diastolic potential was -50 mV.

**Figure 2.** A cartoon showing the contribution of different currents during the SAN action potential and their contribution to the pacemaker depolarisation and action potential repolarisation.

**Figure 3.** A cartoon showing the role of  $K_{ATP}$  channels in SAN function.

## Reference List

1. Jouven, X., Empana, J. P., Schwartz, P. J., Desnos, M., Courbon, D., and Ducimetiere, P. (2005) Heart-rate profile during exercise as a predictor of sudden death. *N. Engl. J. Med.* **352**, 1951-1958
2. Swedberg, K., Komajda, M., Bohm, M., Borer, J. S., Ford, I., Dubost-Brama, A., Lerebours, G., and Tavazzi, L. (2010) Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *Lancet* **376**, 875-885
3. DiFrancesco, D. (2010) The role of the funny current in pacemaker activity. *Circ. Res.* **106**, 434-446
4. Lakatta, E. G., Maltsev, V. A., and Vinogradova, T. M. (2010) A coupled SYSTEM of intracellular Ca<sup>2+</sup> clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ. Res.* **106**, 659-673
5. Boyett, M. R., Honjo, H., and Kodama, I. (2000) The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc. Res.* **47**, 658-687
6. Shibata, N., Inada, S., Mitsui, K., Honjo, H., Yamamoto, M., Niwa, R., Boyett, M. R., and Kodama, I. (2001) Pacemaker shift in the rabbit sinoatrial node in response to vagal nerve stimulation. *Exp. Physiol* **86**, 177-184
7. Mesirca, P., Torrente, A. G., and Mangoni, M. E. (2015) Functional role of voltage gated Ca(2+) channels in heart automaticity. *Front Physiol* **6**, 19
8. Torrente, A. G., Mesirca, P., Neco, P., Rizzetto, R., Dubel, S., Barrere, C., Sinegger-Brauns, M., Striessnig, J., Richard, S., Nargeot, J., Gomez, A. M., and Mangoni, M. E. (2016) L-type Cav1.3 channels regulate ryanodine receptor-dependent Ca<sup>2+</sup> release during sino-atrial node pacemaker activity. *Cardiovasc. Res.* **109**, 451-461
9. Priest, B. T. and McDermott, J. S. (2015) Cardiac ion channels. *Channels (Austin. )* **9**, 352-359
10. Lei, M., Jones, S. A., Liu, J., Lancaster, M. K., Fung, S. S., Dobrzynski, H., Camelliti, P., Maier, S. K., Noble, D., and Boyett, M. R. (2004) Requirement of neuronal- and cardiac-type sodium channels for murine sinoatrial node pacemaking. *J. Physiol* **559**, 835-848
11. Chambers, J. C., Zhao, J., Terracciano, C. M., Bezzina, C. R., Zhang, W., Kaba, R., Navaratnarajah, M., Lotlikar, A., Sehmi, J. S., Kooner, M. K., Deng, G., Siedlecka, U., Parasramka, S., El-Hamamsy, I., Wass, M. N., Dekker, L. R., de Jong, J. S., Sternberg, M. J., McKenna, W., Severs, N. J., de, S. R., Wilde, A. A., Anand, P., Yacoub, M., Scott, J., Elliott, P., Wood, J. N., and Kooner, J. S. (2010) Genetic variation in SCN10A influences cardiac conduction. *Nat. Genet.* **42**, 149-152
12. Mangoni, M. E., Couette, B., Bourinet, E., Platzer, J., Reimer, D., Striessnig, J., and

- Nargeot, J. (2003) Functional role of L-type Cav1.3 Ca<sup>2+</sup> channels in cardiac pacemaker activity. *Proc. Natl. Acad. Sci. U. S. A* **100**, 5543-5548
13. Mangoni, M. E., Traboulsie, A., Leoni, A. L., Couette, B., Marger, L., Le, Q. K., Kupfer, E., Cohen-Solal, A., Vilar, J., Shin, H. S., Escande, D., Charpentier, F., Nargeot, J., and Lory, P. (2006) Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels. *Circ. Res.* **98**, 1422-1430
  14. Vinogradova, T. M., Tagirova, S. S., and Lakatta, E. G. (2018) Unique Ca(2+)-Cycling Protein Abundance and Regulation Sustains Local Ca(2+) Releases and Spontaneous Firing of Rabbit Sinoatrial Node Cells. *Int. J. Mol. Sci.* **19**,
  15. Bogdanov, K. Y., Maltsev, V. A., Vinogradova, T. M., Lyashkov, A. E., Spurgeon, H. A., Stern, M. D., and Lakatta, E. G. (2006) Membrane potential fluctuations resulting from submembrane Ca<sup>2+</sup> releases in rabbit sinoatrial nodal cells impart an exponential phase to the late diastolic depolarization that controls their chronotropic state. *Circ. Res.* **99**, 979-987
  16. Sanders, L., Rakovic, S., Lowe, M., Mattick, P. A., and Terrar, D. A. (2006) Fundamental importance of Na<sup>+</sup>-Ca<sup>2+</sup> exchange for the pacemaking mechanism in guinea-pig sino-atrial node. *J. Physiol* **571**, 639-649
  17. Philipson, K. D. and Nishimoto, A. Y. (1980) Na<sup>+</sup>-Ca<sup>2+</sup> exchange is affected by membrane potential in cardiac sarcolemmal vesicles. *J. Biol. Chem.* **255**, 6880-6882
  18. Cheng, H., Lederer, W. J., and Cannell, M. B. (1993) Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* **262**, 740-744
  19. Bogdanov, K. Y., Vinogradova, T. M., and Lakatta, E. G. (2001) Sinoatrial nodal cell ryanodine receptor and Na(+)-Ca(2+) exchanger: molecular partners in pacemaker regulation. *Circ. Res.* **88**, 1254-1258
  20. Rigg, L., Heath, B. M., Cui, Y., and Terrar, D. A. (2000) Localisation and functional significance of ryanodine receptors during beta-adrenoceptor stimulation in the guinea-pig sino-atrial node. *Cardiovasc. Res.* **48**, 254-264
  21. Huser, J., Blatter, L. A., and Lipsius, S. L. (2000) Intracellular Ca<sup>2+</sup> release contributes to automaticity in cat atrial pacemaker cells. *J. Physiol* **524 Pt 2**, 415-422
  22. Sirenko, S., Yang, D., Li, Y., Lyashkov, A. E., Lukyanenko, Y. O., Lakatta, E. G., and Vinogradova, T. M. (2013) Ca(2+)-dependent phosphorylation of Ca(2+) cycling proteins generates robust rhythmic local Ca(2+) releases in cardiac pacemaker cells. *Sci. Signal.* **6**, ra6
  23. Vinogradova, T. M., Brochet, D. X., Sirenko, S., Li, Y., Spurgeon, H., and Lakatta, E. G. (2010) Sarcoplasmic reticulum Ca<sup>2+</sup> pumping kinetics regulates timing of local Ca<sup>2+</sup> releases and spontaneous beating rate of rabbit sinoatrial node pacemaker cells. *Circ. Res.* **107**, 767-775
  24. Andersson, K. B., Birkeland, J. A., Finsen, A. V., Louch, W. E., Sjaastad, I., Wang, Y.,

- Chen, J., Molkentin, J. D., Chien, K. R., Sejersted, O. M., and Christensen, G. (2009) Moderate heart dysfunction in mice with inducible cardiomyocyte-specific excision of the *Serca2* gene. *J. Mol. Cell Cardiol.* **47**, 180-187
25. Li, Y., Sirenko, S., Riordon, D. R., Yang, D., Spurgeon, H., Lakatta, E. G., and Vinogradova, T. M. (2016) CaMKII-dependent phosphorylation regulates basal cardiac pacemaker function via modulation of local Ca<sup>2+</sup> releases. *Am. J. Physiol Heart Circ. Physiol* **311**, H532-H544
  26. Groenke, S., Larson, E. D., Alber, S., Zhang, R., Lamp, S. T., Ren, X., Nakano, H., Jordan, M. C., Karagueuzian, H. S., Roos, K. P., Nakano, A., Proenza, C., Philipson, K. D., and Goldhaber, J. I. (2013) Complete atrial-specific knockout of sodium-calcium exchange eliminates sinoatrial node pacemaker activity. *PLoS. One.* **8**, e81633
  27. Zaritsky, J. J., Redell, J. B., Tempel, B. L., and Schwarz, T. L. (2001) The consequences of disrupting cardiac inwardly rectifying K(+) current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. *J. Physiol* **533**, 697-710
  28. Liu, G. X., Derst, C., Schlichthorl, G., Heinen, S., Seebohm, G., Bruggemann, A., Kummer, W., Veh, R. W., Daut, J., and Preisig-Muller, R. (2001) Comparison of cloned Kir2 channels with native inward rectifier K<sup>+</sup> channels from guinea-pig cardiomyocytes. *J. Physiol* **532**, 115-126
  29. Noma, A. and Trautwein, W. (1978) Relaxation of the ACh-induced potassium current in the rabbit sinoatrial node cell. *Pflügers Archiv* **377**, 193-200
  30. Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D. E. (1995) The G-protein-gated atrial K<sup>+</sup> channel IKACH is a heteromultimer of two inwardly rectifying K(+)-channel proteins. *Nature* **374**, 135-141
  31. Corey, S. and Clapham, D. E. (1998) Identification of native atrial G-protein-regulated inwardly rectifying K<sup>+</sup> (GIRK4) channel homomultimers. *Journal of Biological Chemistry* **273**, 27499-27504
  32. Fleischmann, B. K., Duan, Y., Fan, Y., Schoneberg, T., Ehlich, A., Lenka, N., Viatchenko-Karpinski, S., Pott, L., Hescheler, J., and Fakler, B. (2004) Differential subunit composition of the G protein-activated inward-rectifier potassium channel during cardiac development. *J. Clin. Invest* **114**, 994-1001
  33. Beckmann, C., Rinne, A., Littwitz, C., Mintert, E., Bosche, L. I., Kienitz, M. C., Pott, L., and Bender, K. (2008) G protein-activated (GIRK) current in rat ventricular myocytes is masked by constitutive inward rectifier current (I(K1)). *Cell Physiol Biochem.* **21**, 259-268
  34. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M., and Hille, B. (1985) GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature* **317**, 536-538
  35. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) The

beta gamma subunits of GTP-binding proteins activate the muscarinic K<sup>+</sup> channel in heart. *Nature* **325**, 321-326

36. Ford, C. E., Skiba, N. P., Bae, H., Daaka, Y., Reuveny, E., Shekter, L. R., Rosal, R., Weng, G., Yang, C.-S., Iyengar, R., Miller, R. J., Jan, L. Y., Lefkowitz, R. J., and Hamm, H. E. (1998) Molecular basis for interactions of G protein  $\beta\gamma$  subunits with effectors. *Science* **280**, 1271-1274
37. Slesinger, P. A., Reuveny, E., Jan, Y. N., and Jan, L. Y. (1995) Identification of structural elements involved in G protein gating of the GIRK1 potassium channel. *Neuron* **15**, 1145-1156
38. Whorton, M. R. and MacKinnon, R. (2011) Crystal structure of the mammalian GIRK2 K<sup>+</sup> channel and gating regulation by G proteins, PIP<sub>2</sub>, and sodium. *Cell* **147**, 199-208
39. Ho, I. H. M. and Murrell-Lagnado, R. D. (1999) Molecular determinants for sodium-dependent activation of G protein-gated K<sup>+</sup> channels. *Journal of Biological Chemistry* **274**, 8639-8648
40. Zhang, H., He, C., Yan, X., Mirshahi, T., and Logothetis, D. E. (1999) Activation of inwardly rectifying K<sup>+</sup> channels by distinct PtdIns(4,5)P<sub>2</sub> interactions. *Nat. Cell Biol.* **1**, 183-188
41. Leaney, J. L., Milligan, G., and Tinker, A. (2000) The G protein  $\alpha$  subunit has a key role in determining the specificity of coupling to, but not the activation of G protein-gated inwardly rectifying K<sup>+</sup> channels. *J. Biol. Chem.* **275**, 921-929
42. Riven, I., Iwanir, S., and Reuveny, E. (2006) GIRK channel activation involves a local rearrangement of a preformed G protein channel complex. *Neuron* **51**, 561-573
43. Clancy, S. M., Fowler, C. E., Finley, M., Suen, K. F., Arrabit, C., Berton, F., Kosaza, T., Casey, P. J., and Slesinger, P. A. (2005) Pertussis-toxin-sensitive Galpha subunits selectively bind to C-terminal domain of neuronal GIRK channels: evidence for a heterotrimeric G-protein-channel complex. *Mol. Cell Neurosci.* **28**, 375-389
44. Peleg, S., Varon, D., Ivanina, T., Dessauer, C. W., and Dascal, N. (2002) G( $\alpha$ )(i) controls the gating of the G protein-activated K(+) channel, GIRK. *Neuron* **33**, 87-99
45. Ivanina, T., Varon, D., Peleg, S., Rishal, I., Porozov, Y., Dessauer, C. W., Keren-Raifman, T., and Dascal, N. (2004) Galpha1 and Galpha3 differentially interact with, and regulate, the G protein-activated K<sup>+</sup> channel. *J. Biol. Chem.* **279**, 17260-17268
46. Nobles, M., Benians, A., and Tinker, A. (2005) Heterotrimeric G-proteins precouple with G-protein coupled receptors in living cells. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 18706-18711
47. Fisher, J. T., Vincent, S. G., Gomeza, J., Yamada, M., and Wess, J. (2004) Loss of vagally mediated bradycardia and bronchoconstriction in mice lacking M<sub>2</sub> or M<sub>3</sub> muscarinic acetylcholine receptors. *FASEB J.* **18**, 711-713
48. Wickman, K., Nemeč, J., Gendler, S. J., and Clapham, D. E. (1998) Abnormal heart

rate regulation in GIRK4 knockout mice. *Neuron* **20**, 103-114

49. Zuberi, Z., Birnbaumer, L., and Tinker, A. (2008) The role of inhibitory heterotrimeric G-proteins in the control of in-vivo heart rate dynamics. *Am. J. Physiol Regul. Integr. Comp Physiol* **295**, R1822-R1830
50. Gehrman, J., Meister, M., Maguire, C. T., Martins, D. C., Hammer, P. E., Neer, E. J., Berul, C. I., and Mende, U. (2002) Impaired parasympathetic heart rate control in mice with a reduction of functional G protein betagamma-subunits. *Am. J. Physiol Heart Circ. Physiol* **282**, H445-H456
51. DiFrancesco, D. and Tromba, C. (1987) Acetylcholine inhibits activation of the cardiac hyperpolarizing-activated current, *if*. *Pflugers Arch.* **410**, 139-142
52. Accili, E. A., Redaelli, G., and DiFrancesco, D. (1998) Two distinct pathways of muscarinic current responses in rabbit sino-atrial node myocytes. *Pflugers Arch.* **437**, 164-167
53. Boyett, M. R., Kodama, I., Honjo, H., Arai, A., Suzuki, R., and Toyama, J. (1995) Ionic basis of the chronotropic effect of acetylcholine on the rabbit sinoatrial node. *Cardiovasc. Res.* **29**, 867-878
54. Mesirca, P., Marger, L., Toyoda, F., Rizzetto, R., Audoubert, M., Dubel, S., Torrente, A. G., DiFrancesco, M. L., Muller, J. C., Leoni, A. L., Couette, B., Nargeot, J., Clapham, D. E., Wickman, K., and Mangoni, M. E. (2013) The G-protein-gated K<sup>+</sup> channel, IKACH, is required for regulation of pacemaker activity and recovery of resting heart rate after sympathetic stimulation. *J. Gen. Physiol* **142**, 113-126
55. Savonen, K. P., Lakka, T. A., Laukkanen, J. A., Halonen, P. M., Rauramaa, T. H., Salonen, J. T., and Rauramaa, R. (2006) Heart rate response during exercise test and cardiovascular mortality in middle-aged men. *Eur. Heart J.* **27**, 582-588
56. Yang, J., Huang, J., Maity, B., Gao, Z., Lorca, R. A., Gudmundsson, H., Li, J., Stewart, A., Swaminathan, P. D., Ibeawuchi, S. R., Shepherd, A., Chen, C. K., Kutschke, W., Mohler, P. J., Mohapatra, D. P., Anderson, M. E., and Fisher, R. A. (2010) RGS6, a modulator of parasympathetic activation in heart. *Circ. Res.* **107**, 1345-1349
57. Posokhova, E., Wydeven, N., Allen, K. L., Wickman, K., and Martemyanov, K. A. (2010) RGS6/Gss5 complex accelerates IKACH gating kinetics in atrial myocytes and modulates parasympathetic regulation of heart rate. *Circ. Res.* **107**, 1350-1354
58. Ramirez, J., Duijvenboden, S. V., Ntalla, I., Mifsud, B., Warren, H. R., Tzanis, E., Orini, M., Tinker, A., Lambiase, P. D., and Munroe, P. B. (2018) Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nat. Commun.* **9**, 1947
59. Mesirca, P., Alig, J., Torrente, A. G., Muller, J. C., Marger, L., Rollin, A., Marquilly, C., Vincent, A., Dubel, S., Bidaud, I., Fernandez, A., Seniuk, A., Engeland, B., Singh, J., Miquerol, L., Ehmke, H., Eschenhagen, T., Nargeot, J., Wickman, K., Isbrandt, D., and Mangoni, M. E. (2014) Cardiac arrhythmia induced by genetic silencing of 'funny' (f) channels is rescued by GIRK4 inactivation. *Nat. Commun.* **5**, 4664

60. Mesirca, P., Bidaud, I., Briec, F., Evain, S., Torrente, A. G., Le, Q. K., Leoni, A. L., Baudot, M., Marger, L., Chung You, C. A., Nargeot, J., Striessnig, J., Wickman, K., Charpentier, F., and Mangoni, M. E. (2016) G protein-gated IKACH channels as therapeutic targets for treatment of sick sinus syndrome and heart block. *Proc. Natl. Acad. Sci. U. S. A* **113**, E932-E941
61. Lei, M. and Brown, H. F. (1996) Two components of the delayed rectifier potassium current, IK, in rabbit sino-atrial node cells. *Exp. Physiol* **81**, 725-741
62. Smith, P. L., Baukrowitz, T., and Yellen, G. (1996) The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* **379**, 833-836
63. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* **269**, 92-95
64. Abbott, G. W., Sesti, F., Splawski, I., Buck, M. E., Lehmann, M. H., Timothy, K. W., Keating, M. T., and Goldstein, S. A. (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* **97**, 175-187
65. Verheijck, E. E., Wilders, R., and Bouman, L. N. (2002) Atrio-sinus interaction demonstrated by blockade of the rapid delayed rectifier current. *Circulation* **105**, 880-885
66. Clark, R. B., Mangoni, M. E., Lueger, A., Couette, B., Nargeot, J., and Giles, W. R. (2004) A rapidly activating delayed rectifier K<sup>+</sup> current regulates pacemaker activity in adult mouse sinoatrial node cells. *Am. J. Physiol Heart Circ. Physiol* **286**, H1757-H1766
67. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) K(V)LQT1 and IsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature* **384**, 78-80
68. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature* **384**, 80-83
69. Davies, M. P., Freeman, L. C., and Kass, R. S. (1996) Dual actions of the novel class III antiarrhythmic drug NE-10064 on delayed potassium channel currents in guinea pig ventricular and sinoatrial node cells. *J. Pharmacol. Exp. Ther.* **276**, 1149-1154
70. Lei, M., Brown, H. F., and Terrar, D. A. (2000) Modulation of delayed rectifier potassium current, iK, by isoprenaline in rabbit isolated pacemaker cells. *Exp. Physiol* **85**, 27-35
71. Bennett, P. B. and Begenisich, T. B. (1987) Catecholamines modulate the delayed rectifying potassium current (IK) in guinea pig ventricular myocytes. *Pflugers Arch.* **410**, 217-219
72. Marx, S. O., Kurokawa, J., Reiken, S., Motoike, H., D'Armiento, J., Marks, A. R., and Kass, R. S. (2002) Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*



73. Kurokawa, J., Chen, L., and Kass, R. S. (2003) Requirement of subunit expression for cAMP-mediated regulation of a heart potassium channel. *Proc. Natl. Acad. Sci. U. S. A* **100**, 2122-2127
74. Sanguinetti, M. C., Jurkiewicz, N. K., Scott, A., and Siegl, P. K. (1991) Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. *Circ. Res.* **68**, 77-84
75. Harmati, G., Banyasz, T., Barandi, L., Szentandrassy, N., Horvath, B., Szabo, G., Szentmiklosi, J. A., Szenasi, G., Nanasi, P. P., and Magyar, J. (2011) Effects of beta-adrenoceptor stimulation on delayed rectifier K(+) currents in canine ventricular cardiomyocytes. *Br. J. Pharmacol.* **162**, 890-896
76. Xie, Y., Ding, W. G., and Matsuura, H. (2015) Ca<sup>2+</sup>/calmodulin potentiates I Ks in sinoatrial node cells by activating Ca<sup>2+</sup>/calmodulin-dependent protein kinase II. *Pflugers Arch.* **467**, 241-251
77. Unudurthi, S. D., Wu, X., Qian, L., Amari, F., Onal, B., Li, N., Makara, M. A., Smith, S. A., Snyder, J., Fedorov, V. V., Coppola, V., Anderson, M. E., Mohler, P. J., and Hund, T. J. (2016) Two-Pore K<sup>+</sup> Channel TREK-1 Regulates Sinoatrial Node Membrane Excitability. *J. Am. Heart Assoc.* **5**, e002865
78. Bao, L., Kefaloyianni, E., Lader, J., Hong, M., Morley, G., Fishman, G. I., Sobie, E. A., and Coetzee, W. A. (2011) Unique properties of the ATP-sensitive K(+) channel in the mouse ventricular cardiac conduction system. *Circ. Arrhythm. Electrophysiol.* **4**, 926-935
79. Flagg, T. P., Kurata, H. T., Masia, R., Caputa, G., Magnuson, M. A., Lefer, D. J., Coetzee, W. A., and Nichols, C. G. (2008) Differential structure of atrial and ventricular KATP: atrial KATP channels require SUR1. *Circ. Res.* **103**, 1458-1465
80. Inagaki, N., Gonoi, T., Clement, J. P. 4., Namba, N., Inazawa, J., Gonzalez, G., Aguilar Bryan, L., Seino, S., and Bryan, J. (1995) Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270**, 1166-1170
81. Inagaki, N., Gonoi, T., Clement, J. P., Wang, C. Z., Aguilar Bryan, L., Bryan, J., and Seino, S. (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K<sup>+</sup> channels. *Neuron* **16**, 1011-1017
82. Aguilar Bryan, L., Nichols, C. G., Wechsler, S. W., Clement, J. P. 4., Boyd, A. E., Gonzalez, G., Herrera Sosa, H., Nguy, K., Bryan, J., and Nelson, D. A. (1995) Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* **268**, 423-426
83. Li, N., Wu, J. X., Ding, D., Cheng, J., Gao, N., and Chen, L. (2017) Structure of a Pancreatic ATP-Sensitive Potassium Channel. *Cell* **168**, 101-110
84. Martin, G. M., Yoshioka, C., Rex, E. A., Fay, J. F., Xie, Q., Whorton, M. R., Chen, J. Z., and Shyng, S. L. (2017) Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. *Elife.* **6**,

85. Lee, K. P. K., Chen, J., and MacKinnon, R. (2017) Molecular structure of human KATP in complex with ATP and ADP. *Elife*. **6**,
86. Noma, A. (1983) ATP-regulated K<sup>+</sup> channels in cardiac muscle. *Nature* **305**, 147-148
87. Tinker, A., Aziz, Q., and Thomas, A. (2014) The role of ATP-sensitive potassium channels in cellular function and protection in the cardiovascular system. *Br. J. Pharmacol.* **171**, 12-23
88. Zingman, L. V., Hodgson, D. M., Bast, P. H., Kane, G. C., Perez-Terzic, C., Gumina, R. J., Pucar, D., Bienengraeber, M., Dzeja, P. P., Miki, T., Seino, S., Alekseev, A. E., and Terzic, A. (2002) Kir6.2 is required for adaptation to stress. *Proc. Natl. Acad. Sci. U. S. A* **99**, 13278-13283
89. Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M., and Seino, S. (1995) Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J. Biol. Chem.* **270**, 5691-5694
90. Beech, D. J., Zhang, H., Nakao, K., and Bolton, T. B. (1993) K channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. *Br. J. Pharmacol.* **110**, 573-582
91. Cui, Y., Tran, S., Tinker, A., and Clapp, L. H. (2002) The molecular composition of K(ATP) channels in human pulmonary artery smooth muscle cells and their modulation by growth. *Am. J. Respir. Cell Mol. Biol.* **26**, 135-143
92. Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y., and Kurachi, Y. (1997) Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K<sup>+</sup> channel. *J. Physiol* **499** ( Pt 3), 715-720
93. Aziz, Q., Thomas, A. M., Gomes, J., Ang, R., Sones, W. R., Li, Y., Ng, K. E., Gee, L., and Tinker, A. (2014) The ATP-Sensitive Potassium Channel Subunit, Kir6.1, in Vascular Smooth Muscle Plays a Major Role in Blood Pressure Control. *Hypertension* **64**, 523-529
94. Fukuzaki, K., Sato, T., Miki, T., Seino, S., and Nakaya, H. (2008) Role of sarcolemmal ATP-sensitive K<sup>+</sup> channels in the regulation of sinoatrial node automaticity: an evaluation using Kir6.2-deficient mice. *J. Physiol* **586**, 2767-2778
95. Han, X., Light, P. E., Giles, W. R., and French, R. J. (1996) Identification and properties of an ATP-sensitive K<sup>+</sup> current in rabbit sino-atrial node pacemaker cells. *J. Physiol* **490** ( Pt 2), 337-350
96. Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H., and Seino, S. (2002) Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat. Med.* **8**, 466-472
97. Aziz, Q., Li, Y., Anderson, N., Ojake, L., Tsisanova, E., and Tinker, A. (2017) Molecular and functional characterization of the endothelial ATP-sensitive potassium channel. *J. Biol. Chem.* **292**, 17587-17597

98. Aziz, Q., Finlay, M., Montaigne, D., Ojake, L., Li, Y., Anderson, N., Ludwig, A., and Tinker, A. (2018) ATP-sensitive potassium channels in the sinoatrial node contribute to heart rate control and adaptation to hypoxia. *J. Biol. Chem.* **293**, 8912-8921
99. Hoesl, E., Stieber, J., Herrmann, S., Feil, S., Tybl, E., Hofmann, F., Feil, R., and Ludwig, A. (2008) Tamoxifen-inducible gene deletion in the cardiac conduction system. *J. Mol. Cell Cardiol.* **45**, 62-69
100. Boyett, M. R., Dobrzynski, H., Lancaster, M. K., Jones, S. A., Honjo, H., and Kodama, I. (2003) Sophisticated architecture is required for the sinoatrial node to perform its normal pacemaker function. *J. Cardiovasc. Electrophysiol.* **14**, 104-106
101. Giblin, J. P., Cui, Y., Clapp, L. H., and Tinker, A. (2002) Assembly limits the pharmacological complexity of ATP-sensitive potassium channels. *J. Biol. Chem.* **277**, 13717-13723
102. Glukhov, A. V., Flagg, T. P., Fedorov, V. V., Efimov, I. R., and Nichols, C. G. (2010) Differential K(ATP) channel pharmacology in intact mouse heart. *J. Mol. Cell Cardiol.* **48**, 152-160
103. Gerlach, A. C. and Antonio, B. M. (2015) Validation of ion channel targets. *Channels (Austin. )* **9**, 376-379
104. Vinogradova, T. M., Lyashkov, A. E., Zhu, W., Ruknudin, A. M., Sirenko, S., Yang, D., Deo, S., Barlow, M., Johnson, S., Caffrey, J. L., Zhou, Y. Y., Xiao, R. P., Cheng, H., Stern, M. D., Maltsev, V. A., and Lakatta, E. G. (2006) High basal protein kinase A-dependent phosphorylation drives rhythmic internal Ca<sup>2+</sup> store oscillations and spontaneous beating of cardiac pacemaker cells. *Circ. Res.* **98**, 505-514
105. Younes, A., Lyashkov, A. E., Graham, D., Sheydina, A., Volkova, M. V., Mitsak, M., Vinogradova, T. M., Lukyanenko, Y. O., Li, Y., Ruknudin, A. M., Boheler, K. R., van, E. J., and Lakatta, E. G. (2008) Ca(2+) -stimulated basal adenylyl cyclase activity localization in membrane lipid microdomains of cardiac sinoatrial nodal pacemaker cells. *J. Biol. Chem.* **283**, 14461-14468
106. Yaniv, Y., Ganesan, A., Yang, D., Ziman, B. D., Lyashkov, A. E., Levchenko, A., Zhang, J., and Lakatta, E. G. (2015) Real-time relationship between PKA biochemical signal network dynamics and increased action potential firing rate in heart pacemaker cells: Kinetics of PKA activation in heart pacemaker cells. *J. Mol. Cell Cardiol.* **86**, 168-178
107. Wilson, A. J., Jabr, R. I., and Clapp, L. H. (2000) Calcium modulation of vascular smooth muscle ATP-sensitive K(+) channels: role of protein phosphatase-2B. *Circ. Res.* **87**, 1019-1025
108. Orié, N. N., Thomas, A. M., Perrino, B. A., Tinker, A., and Clapp, L. H. (2009) Ca<sup>2+</sup>/calcineurin regulation of cloned vascular K ATP channels: crosstalk with the protein kinase A pathway. *Br. J. Pharmacol.* **157**, 554-564
109. Quayle, J. M., Nelson, M. T., and Standen, N. B. (1997) ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev.* **77**, 1165-1232

110. Quinn, K. V., Giblin, J. P., and Tinker, A. (2004) Multisite Phosphorylation Mechanism for Protein Kinase A Activation of the Smooth Muscle ATP-Sensitive K<sup>+</sup> Channel. *Circ. Res.* **94**, 1359-1366
111. Thery, C., Gosselin, B., Lekieffre, J., and Warembourg, H. (1977) Pathology of sinoatrial node. Correlations with electrocardiographic findings in 111 patients. *Am. Heart J.* **93**, 735-740
112. Evans, R. and Shaw, D. B. (1977) Pathological studies in sinoatrial disorder (sick sinus syndrome). *Br. Heart J.* **39**, 778-786
113. Choudhury, M., Boyett, M. R., and Morris, G. M. (2015) Biology of the Sinus Node and its Disease. *Arrhythm. Electrophysiol. Rev.* **4**, 28-34
114. Tellez, J. O., Mczewski, M., Yanni, J., Sutyagin, P., Mackiewicz, U., Atkinson, A., Inada, S., Beresewicz, A., Billeter, R., Dobrzynski, H., and Boyett, M. R. (2011) Ageing-dependent remodelling of ion channel and Ca<sup>2+</sup> clock genes underlying sinoatrial node pacemaking. *Exp. Physiol* **96**, 1163-1178
115. Allah, E. A., Tellez, J. O., Yanni, J., Nelson, T., Monfredi, O., Boyett, M. R., and Dobrzynski, H. (2011) Changes in the expression of ion channels, connexins and Ca<sup>2+</sup>-handling proteins in the sino-atrial node during postnatal development. *Exp. Physiol* **96**, 426-438
116. Opthof, T. (2000) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res.* **45**, 173-176
117. D'Souza, A., Bucchi, A., Johnsen, A. B., Logantha, S. J., Monfredi, O., Yanni, J., Prehar, S., Hart, G., Cartwright, E., Wisloff, U., Dobryznski, H., DiFrancesco, D., Morris, G. M., and Boyett, M. R. (2014) Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat. Commun.* **5**, 3775
118. Yanni, J., Tellez, J. O., Maczewski, M., Mackiewicz, U., Beresewicz, A., Billeter, R., Dobrzynski, H., and Boyett, M. R. (2011) Changes in ion channel gene expression underlying heart failure-induced sinoatrial node dysfunction. *Circ. Heart Fail.* **4**, 496-508
119. Bai, X., Wang, K., Yuan, Y., Li, Q., Dobrzynski, H., Boyett, M. R., Hancox, J. C., and Zhang, H. (2017) Mechanism underlying impaired cardiac pacemaking rhythm during ischemia: A simulation study. *Chaos.* **27**, 093934

Figure 1

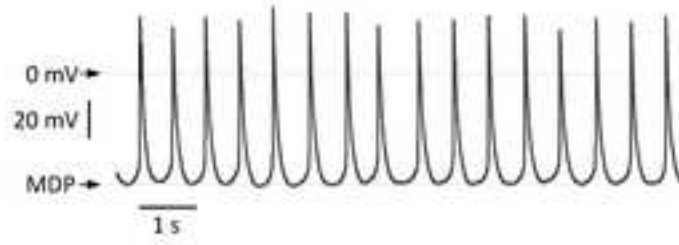


Figure 2

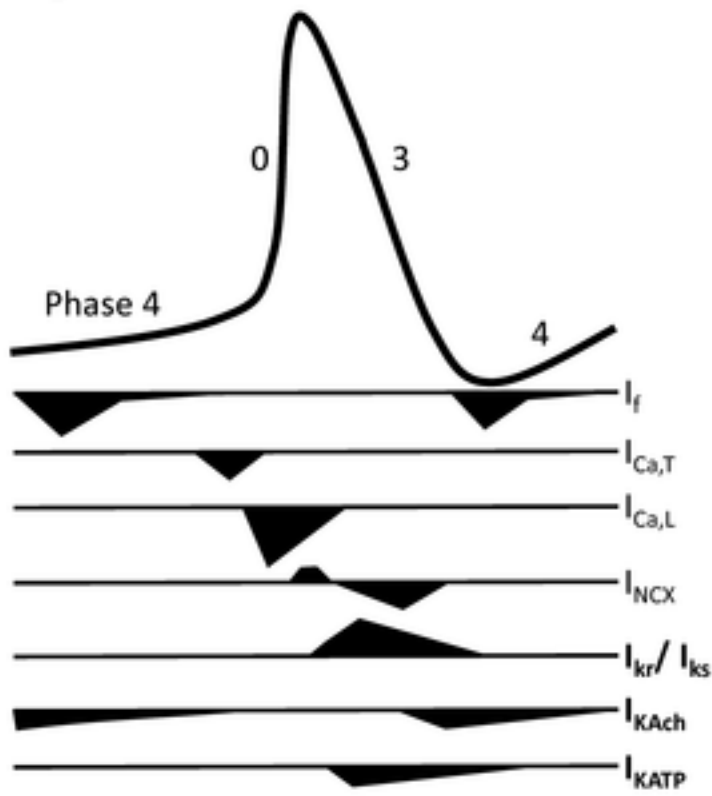
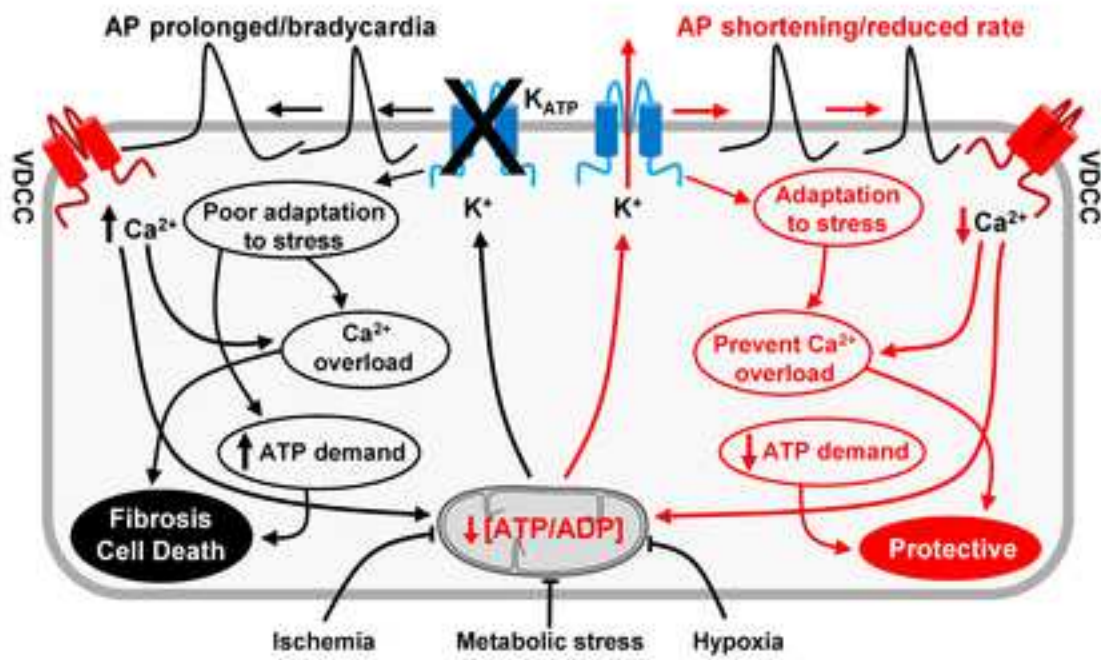


Figure 3



# **Potassium channels in the sinoatrial node and their role in heart rate control**

Qadeer Aziz, Yiwen Li and Andrew Tinker\*

William Harvey Heart Centre,  
Barts & The London School of Medicine & Dentistry,  
Queen Mary, University of London  
Charterhouse Square,  
London, EC1M 6BQ

Tel: 020 7882 5783, E-mail: a.tinker@qmul.ac.uk

\* Corresponding Author

Keywords: Potassium channel, ATP-sensitive potassium channel, heart rate, pacemaking, sinoatrial node.



## **Abstract**

Potassium currents determine the resting membrane potential and govern repolarisation in cardiac myocytes. Here, we review the various currents in the sinoatrial node focussing on their molecular and cellular properties and their role in pacemaking and heart rate control. We also describe how our recent finding of a novel ATP-sensitive potassium channel population in these cells fits into this picture .

## **Introduction**

The regular beating of the heart is generated by intrinsic pacemaker activity in the sinoatrial node (SAN). Heart rate can be easily measured and is known to be associated with disease in a number of epidemiological studies. For example, in Paris Prospective Study I the risk of sudden death increased over three fold (relative risk 3.9) in those with a resting heart rate  $>75$  beats per minute compared to those with one under 60 (1). Furthermore, the increase in heart rate during exercise and the rate of recovery of rate after cessation of exercise were also strongly associated with sudden death (1). Moreover, direct intervention to lower heart rate using drugs with predominant effects on the SAN can improve outcomes in heart failure (2).

The pacemaker action potential has a characteristic morphology with a diastolic depolarisation before a threshold is reached with a consequent action potential. Figure 1 shows this in single mouse SAN cells studied in the current clamp mode of the patch clamp. Exactly how the intrinsic pacemaker clock is generated is controversial. Historically the rhythmical oscillations of membrane potential were thought to be governed by the hyperpolarisation-activated cyclic nucleotide-gated cation channel (also known as the “funny” current,  $I_f$ ) constituted of the product of the HCN genes (largely HCN4) (3). However, published data now show the importance of intracellular calcium cycling

specifically diastolic calcium release from the sarcoplasmic reticulum generating an inward current via the sodium-calcium exchanger (4). It is likely that these two mechanisms are in fact highly interdependent. Furthermore, this oscillatory electrical activity is subject to regulation by the autonomic nervous system and that allows heart rate to increase flexibly with increased demand such as during exercise. In this review, we consider the role of potassium channels in shaping SAN electrophysiology and their part in heart rate control. We also discuss our recent work on a new player in SAN excitability namely ATP-sensitive potassium channels ( $K_{ATP}$ ) constituted of Kir6.1 subunits.

Figure 1 here.

### **An overview of the electrophysiology of the SAN**

The SAN sits in the upper part of the right atrium laterally to the entrance of the superior vena cava. Textbooks show a characteristic pacemaker action potential, however the SAN is actually quite a heterogeneous structure, both morphologically and electrophysiologically (5). The primary pacemaker can also shift location with changes in autonomic tone (6). With this caveat in mind it is helpful to consider the main currents underlying excitability.

The rapid initial depolarisation of the action potential, also known as phase 0, is not as fast as in ventricular tissue and is mediated largely by L-type calcium currents. At the molecular level SAN cells express both  $Ca_v1.2$  and  $Ca_v1.3$ .  $Ca_v1.2$  is the classic L-type channel present throughout the heart that is responsible for calcium entry promoting calcium-induced calcium release from the sarcoplasmic reticulum and in the SA node is responsible for some of the action potential depolarisation (7). In contrast,  $Ca_v1.3$  activates at more hyperpolarised potentials and contributes to pacemaking (8,9). Sodium currents are less

obvious as the main driver of depolarisation. However, tetrodotoxin-resistant and sensitive currents have been described and these may be present in the more peripheral parts of the node adjacent to the atrial tissue (5). Sodium channel mutations in SCN5A result in cardiac conduction disease (10). SCN10A has also been associated with conduction albeit in the atrioventricular node (11). Repolarisation of the action potential is achieved by inactivation of L-type calcium currents and opening of a number of potassium channels. The latter are discussed in more detail below. The SAN action potential lacks a notch and plateau phase (phase 1 and 2) that is characteristic of the ventricular action potential.

The second feature of SAN electrophysiology is the slow diastolic depolarisation also known as the pacemaker potential. In the initial phase this is mediated by sodium influx via  $I_f$ , largely constituted by the HCN4 subunit, and as depolarisation occurs progressively by T-type calcium channels and finally L-type calcium channels, specifically  $Ca_v1.3$ , just prior to threshold and initiation of phase 0.  $Ca_v1.3$  knockout mice have highly unstable pacemaker function with bradycardia and episodes of sinus pauses (12). The major T-type subunit in the adult animal is  $Ca_v3.1$  and knockout mice are bradycardic (13). This slow diastolic depolarisation is opposed by inward rectifier potassium currents and other background currents (see below). Figure 2 summarises the important currents underlying the SAN action potential.

Figure 2 here.

### **How is the rhythmicity of the diastolic depolarisation generated?**

One of the major recent advances has been an appreciation of the importance of intracellular calcium cycling in the genesis of the rhythmicity of the diastolic depolarisation (4,14). In addition to the membrane ion channels detailed above there is also expression of

the sodium-calcium exchanger in the sarcolemma of the SAN myocytes (15,16). The sodium-calcium exchanger transports three sodium ions for one calcium and thus has the potential to generate a net inward current when extruding calcium (17). Calcium is released from the sarcoplasmic reticulum even during diastole when the cell is quiescent and in ventricular cells this is manifest as calcium sparks (18). In SAN myocytes however these local calcium releases are larger involving a number of release sites (19-21). In permeabilised cells the effects of membrane ion channels are removed and in these conditions these events are periodic in contrast to the stochastic nature of sparks in ventricular myocytes (22). The SA node does not have t-tubules and calcium release is sub-sarcolemmal. The timing of the spontaneous calcium release is in the latter part of the diastolic depolarisation and the activation of the inward sodium-calcium exchanger current is exponential ultimately leading to the threshold potential (15,21). The SAN seems to have higher levels of the calcium pump, SERCA2, present in the membranes of the sarcoplasmic reticulum and in inducible knockout mice the heart rate is substantially slowed (23,24). In addition, phospholamban, which in its unphosphorylated state inhibits SERCA2, is reduced in expression and basally phosphorylated compared to the ventricle (25). Pacemaking and spontaneous calcium release are inhibited by cyclopenzoic acid and ryanodine and heart rate is slowed in a mouse with knockout of the sodium-calcium exchanger (16,19,20,26). These observations all support the importance of a fundamental calcium clock in generating the rhythmic oscillator SAN discharge which integrates with the activity of membrane ion channels.

### **Potassium channels and pacemaking**

Given these features of SAN electrophysiology, the opening of potassium channels can contribute in two ways. In the first action potential repolarisation can be accelerated attenuating the contribution of the action potential to the cycle length and thus potentially

increasing heart rate. In contrast an increase in potassium conductance during the slow diastolic depolarisation will increase the maximum diastolic potential and slow the rate of pacemaker depolarisation leading to a slower heart rate. The exact net contribution depends on the properties of the potassium current. If the current predominates around the resting membrane potential, such as with inwardly-rectifying potassium channels, then the net effect will be on the diastolic depolarisation. In contrast if active at more depolarised potentials this could predominantly influence repolarisation. In principle both effects might occur and the net effect on heart rate would be difficult to predict. Modelling in these circumstances might be helpful in aiding the understanding of the physiological consequences of changes in a particular potassium conductance. The regulation of these currents could also be potentially important: increased potassium currents during the diastolic potential leading to heart rate slowing. It is also important that action potential duration adapts by shortening during high heart rates otherwise regular pacemaking would fail. Adrenergic modulation of repolarising potassium conductances is a mechanism to ensure that and this is discussed below.

### **G-protein gated inwardly rectifying potassium (GIRK) currents and other background currents**

All cardiac myocytes express a strong inwardly-rectifying potassium current. In ventricular cells this is known as  $I_{K1}$  and is accounted for by members of the Kir2.0 family of inward rectifiers (9,27,28). In contrast in the SAN and atrial cells the current is less strongly inwardly-rectifying but is characteristically increased by muscarinic agonists such as acetylcholine and carbachol (" $I_{KACH}$ ") (29). This current is a heteromultimer of Kir3.0 subunits that in cardiac cells is constituted of Kir3.1 and Kir3.4 with perhaps some homomultimers of Kir3.4 (30-32). The differential expression is not absolute for example there are some data indicating expression of  $I_{KACH}$  in the ventricle (33).

Activation of  $I_{KACH}$  is characteristically inhibited by muscarinic antagonists and activation is inhibited by pertussis toxin implicating heterotrimeric G-proteins in the regulation (34). After some controversy, it became clear that it was the  $G_{\beta\gamma}$  subunit, not the inhibitory  $G_{\alpha}$ , that directly activated the channel complex and in many ways this is now the paradigmatic example of modulation of an effector by  $G_{\beta\gamma}$  (35,36). The domains on the Kir3.0 subunits that bind the  $G_{\beta\gamma}$  subunits and also the key residues on  $G_{\beta\gamma}$  have been mapped (36,37). There are crystal structures of the channel complex with and without  $G_{\beta\gamma}$  subunits bound (38). Anionic phospholipids particularly phosphatidylinositol-(4,5)-bisphosphate and sodium are known to be key modulators of the gating and a number of site-directed mutagenesis studies together with structural work have suggested models predicting how this might occur (38-40). However the inhibitory G-protein  $\alpha$  subunit also participates in determining the selectivity of activation but not directly in activation itself (41). There are strands of evidence pointing to models in which the inhibitory heterotrimeric G-protein is complexed with the channel and receptor prior to activation (42-46).

The pathway delineated by genetic, physiological and pharmacological studies involved the activation of  $M_2$  muscarinic receptors and the dissociation of the inhibitory heterotrimer with the  $G_{\beta\gamma}$  activating GIRK channels in the SAN (47-50). However the GTP bound inhibitory  $G_{\alpha}$  subunit can also inhibit adenylate cyclase and reduce levels of cAMP reducing  $I_f$  (51). Thus there are two possible mechanisms for the reduction of heart rate via the parasympathetic arm of the autonomic nervous system: one involving GIRK and another via  $I_f$ . The relative importance of these has been debated over the years. Initially, low receptor occupancy was associated with  $I_f$  inhibition whilst higher receptor occupancy was needed for  $I_{KACH}$  activation (52). The cloning of the Kir3.0 channel subunits enabled the development of mice with global genetic deletion of GIRK4 (*knj5*). These mice together with other experimental observations clearly implicate GIRK channels and  $I_{KACH}$  in heart rate regulation

in physiological conditions (48,53).

GIRK channels are also important in the recovery of heart rate: GIRK4 knockout mice when exercised show a delay in the time taken to return to the resting heart rate (54). This is intriguing as a delayed recovery of heart rate with exercise is associated with poor outcomes (55). Regulator of G-protein signalling 6 (RGS6) is also critical to heart rate regulation (56,57) and acts as the predominant GTPase activating protein for inhibitory G-proteins in the SAN. The net result of this is to inhibit signalling but it also delays the recovery to the inactive state once the agonist is withdrawn. Intriguingly, in our recent genome wide association study in man examining the genetic architecture of heart rate response to exercise we implicated the genes encoding M<sub>2</sub> muscarinic receptor, acetylcholinesterase and RGS6 in determining recovery responses after exercise (58). Furthermore, genetic deletion or pharmacological inhibition of GIRK4 may also ameliorate the bradycardic and proarrhythmic effects of HCN4 and Ca<sub>v</sub>1.3 deletion (59,60).

### **Repolarising currents**

The major repolarising currents in ventricular myocytes are I<sub>Kr</sub> and I<sub>Ks</sub> and both components are also present in SAN myocytes (9,61). I<sub>Kr</sub> is named as such because it is rapidly activating and the currents are characteristically inwardly rectifying due to its unique inactivation properties (62). I<sub>Kr</sub> is composed of hERG (human ether-a-go-go related gene) and possibly a β subunit of the KCNE family though this is still controversial (63,64). I<sub>Kr</sub> is an important current in SAN cells (65,66). For example, the inhibitor E-4031 leads to significant perturbation of the SAN action potential and dofetilide (another blocker) slows pacemaking (61). In contrast, I<sub>Ks</sub> is characteristically activates slowly and the channel complex is formed by KCNQ1 (Kv7.1) and the β subunit KCNE1 (67,68). There may be some species differences in the relative magnitudes of I<sub>Kr</sub> and I<sub>Ks</sub> but I<sub>Ks</sub> is also clearly present in the SAN

(69,70). One key property of these currents, in particular  $I_{Ks}$ , is the augmentation by  $\beta$  adrenergic signalling: it is key in shortening the action potential duration during high heart rates (70,71). Activation of  $\beta$  receptors leads to PKA stimulation and direct phosphorylation of residues S27 and S92 in the channel N-terminus (72). The increase in current requires KCNQ1 to be associated with KCNE1 and is dependent on a protein kinase A anchoring protein (AKAP) *AKAP9* (72,73). Unlike  $I_{Ks}$  regulation, there is no agreement on whether  $I_{Kr}$  currents can be increased by  $\beta$  adrenoreceptor modulation (74,75). Uniquely in the SAN, calcium-calmodulin kinase II may activate  $I_{Ks}$  and intriguingly this could affect calcium handling and channel modulation too (76).

Background potassium currents carried by twin pore channels are difficult to investigate given their poor pharmacology. However a recent study using cardiac-specific TREK1 knockout mice revealed that these mice were bradycardic and predisposed to sinus arrest (77). Thus it seems likely that TREK1 also contributes to repolarisation.

### **$K_{ATP}$ channels underlie repolarising currents in the SA node**

ATP-sensitive potassium channels are widely distributed in the heart. In general the focus has largely been on channels present in the ventricle but characteristic currents can also be recorded in the atria and in the conduction system including the SAN (78,79). The defining property of these channels is their sensitivity to intracellular nucleotides and they are activated by declining ATP levels and/or increasing magnesium ADP levels. The channel complex is made up of four pore-forming inwardly-rectifying Kir6.0 subunits (Kir6.1, Kir6.2) together with four regulatory sulphonylurea receptors (SUR1, SUR2A, SUR2B) (80-82). We now understand these channels well as molecular machines underpinned by exhaustive mutagenesis work and more recently by structural studies. Cryo-electron microscopy has shown how ATP binds to the pore-forming subunit and how MgADP



interacts with the nucleotide binding domains in the sulphonylurea receptor (83-85). Despite the initial description of these channels in the heart (86) there are still questions as to their exact physiological role. Kir6.2 is thought to underlie the classic current present in pancreatic  $\beta$  cells and ventricular cardiomyocytes (87). Kir6.2 global knockout mice are unable to tolerate high intensity exercise partly due to impaired cardiac performance (88).

There has been comparatively less work on the Kir6.1 subunit though it has been known since it was first cloned that it is widely expressed (89). Furthermore, there exists, particularly in smooth muscle, a  $K_{ATP}$  channel with a lower single-channel conductance (35 pS vs 70 pS) with an absolute dependence on cellular nucleotide diphosphates for channel opening. In some papers this led to the channel being called a “ $K_{NDP}$ ” (90). In addition, ATP seems less potent in causing channel inhibition (90,91). This current is recapitulated in heterologous expression systems by the co-expression of Kir6.1 and SUR2B (92) and smooth selective deletion of Kir6.1 (*kcnj8*) in mice abolished the current present in isolated single smooth muscle cells (93).

$K_{ATP}$  currents are present in single isolated SAN cells and they were abolished in cells isolated from the Kir6.2 knockout mouse (94). Interestingly single channel studies revealed a single channel conductance of  $\sim 50$  pS in contrast to the situation in the ventricle where it is closer to 70-80 pS (95). There are reasons to believe that pacemaker function may also be influenced by  $K_{ATP}$  channels containing the Kir6.1 subunit. Mice with global genetic deletion of Kir6.1 develop SAN failure and also heart block which leads to sudden death (93,96). In our own studies we have studied mice with selective deletion of Kir6.1 in vascular smooth muscle or endothelium (93,97). These mice do not show the rhythm disturbance even with provocations that might provoke vasospasm and suggest by exclusion a potential role for Kir6.1 in the SA and atrioventricular node (93,97).

We have explored this question in the mouse using *cre/loxP* technology (98). We

made use of a cre driver line that allows selective deletion of genes within the adult conduction system on the addition of tamoxifen (98,99). The resulting knockout mice are labelled as cKO. We first isolated  $K_{ATP}$  currents in single SAN cells and find that only approximately half the cells contain currents (98). This is not surprising given the heterogeneous electrophysiological nature of the SAN (100). Importantly this number of cells was significantly reduced in cKO mice (98). The pharmacology of the response is also interesting with diazoxide activating and tolbutamide inhibiting and this profile is more typical of SUR1 than SUR2 (101). In murine atrial myocytes SUR1 underpins a significant population of  $K_{ATP}$  channels (102). Furthermore when action potentials were measured in single SAN cells, there was delayed repolarisation in cKO cells resulting in prolonged cycle length (98).

The murine model also allows the investigation of the *in-vivo* physiological consequences of the Kir6.1 deletion (103). Using implanted telemetry probes, we measured heart rate and ECG parameters in awake mice over a prolonged period. cKO mice are bradycardic and in some mice there were episodes of sinus arrest which were not seen in littermate controls (98). Furthermore, there was an indication of atrioventricular nodal dysfunction with an increase of the PR interval on the ECG (98). We also saw some modest pathological changes with fibrosis in the SA node in a few of the cKO mice (98). The phenotype is not as pronounced as in the global Kir6.1 knockout animal but the impairment of vascular reactivity and lack of protective responses may accelerate pathological damage (93,98).

In summary, our recent studies show that  $K_{ATP}$  channels are constitutively active in SA nodal cells. The current seems to influence repolarisation predominantly and this results in an increased cycle length. In-vivo this leads to bradycardia but there was also evidence of sinus pauses, heart block and pathological changes in the SA node (98). It is surprising that

there are no effects on the maximum diastolic membrane potential. Kir6.1 is a member of the inwardly rectifying family of potassium channel however the currents are more outwardly rectifying (98). Additionally, it is plausible that there may be some cyclical regulation of  $K_{ATP}$  channel activity during repolarisation perhaps by calcium and/or cAMP and protein kinase A to explain this paradox. Signalling via cAMP seems to be significantly adapted in the SA node. Adenylyl cyclase is constitutively active and this leads to basal protein kinase A activation (104). The SA node in contrast to the ventricle expresses calcium sensitive adenylyl cyclase isoforms (105). PKA activity, and also that of calcium calmodulin dependent kinase II, is necessary for normal pacemaking as inhibitors of these kinases lead to significant slowing of beating (25,106). Given the sensitivity of both adenylyl cyclase and calcium calmodulin dependent protein kinase II to calcium there may also be cyclical variations in activity that, in addition to phosphorylating phospholamban, may also phosphorylate  $K_{ATP}$  channels. On the background of high phosphatase activity this may lead to variations in beat-to-beat channel activity and account for the prominence of currents during repolarisation. It is also known that  $K_{ATP}$  channels can be regulated by calcineurin (107,108). It is known that Kir6.1-containing channels are prominently regulated by hormonal pathways and protein kinases both in heterologous and native tissues (93,109,110).

### **The response of the SAN to hypoxia**

The sinus node is prone to disease in particular sick sinus syndrome which leads to a pathologically slow heart rate and can be accompanied by sinus arrest leading to dizziness and loss of consciousness (111,112). The disease increases in incidence with age and has been attributed to progressive cell loss and fibrosis (111,112). In addition, atrial fibrillation can be accompanied by sinus node disease and “tachy-brady” syndromes are well described (113). However it also clear that there are changes in the expression of relevant pacemaking genes

in the SA node with age. For example HCN4 and SERCA2 decrease in expression and this may be responsible for the decline in intrinsic heart rate with age (14,113-116). It may also be contributing to physiological and pathological adaptations too (117,118). The SAN is supplied by SAN artery which usually arises from the right coronary artery in man though there are anatomical variations. The SA node responds to hypoxia and ischaemia with heart rate slowing and ultimately failure due to exit block from the SA node into the atrium (119). In modelling work, a major component of the response to hypoxia is the opening of  $K_{ATP}$  channels (119). Potential shortening of the action potential duration is offset by increases in calcium current but the membrane potential becomes hyperpolarised and the pacemaker depolarisation is slowed. This results in bradycardia and ultimately SAN failure. Interestingly, these effects are exacerbated by concomitant increased vagal activity (119).

We investigated this issue in a different way (98). Using an *ex-vivo* Langendorff preparation we measured spontaneous firing using a multielectrode array. We measured the sinus node recovery time by overdrive pacing the endogenous activity and then stopping the pacing and measured the time it takes for the SAN to recover spontaneous activity. This measure is used clinically to assess SAN function and in hypoxic conditions in control mice we found it became prolonged (98). This may be a protective response where a reduction in activity helps preserve the viability of SAN myocytes. In contrast in the cKO mice the SAN recovery time did not prolong with hypoxia and under resting conditions was longer. This lack of flexibility may promote SAN damage. The contribution of  $K_{ATP}$  to SAN physiology and pathophysiology is summarised in Figure 3.

[Figure 3 here.](#)

## **Summary**

Potassium channels play key roles in determining SAN repolarisation and the behaviour of the pacemaker potential. In this review we have placed in context the contribution of  $K_{ATP}$  currents to heart rate regulation revealed in our recent studies.

**Sources of funding:** This research was supported by the British Heart Foundation (RG/15/15/31742) and was facilitated by the NIHR Biomedical Research Centre at Barts.

**Disclosures:** None and the authors have no conflicts of interest.

## Figure Legends

**Figure 1.** The figure shows a recording of spontaneous action potentials in a single isolated SAN myocyte recorded in current clamp in the whole cell configuration of the patch clamp. It illustrates the characteristic morphology of the action potential. **The line indicates 0 millivolt and the maximum diastolic potential was -50 mV.**

**Figure 2.** A cartoon showing the contribution of different currents during the SAN action potential and their contribution to the pacemaker depolarisation and action potential repolarisation.

**Figure 3.** A cartoon showing the role of  $K_{ATP}$  channels in SAN function.

## Reference List

1. Jouven, X., Empana, J. P., Schwartz, P. J., Desnos, M., Courbon, D., and Ducimetiere, P. (2005) Heart-rate profile during exercise as a predictor of sudden death. *N. Engl. J. Med.* **352**, 1951-1958
2. Swedberg, K., Komajda, M., Bohm, M., Borer, J. S., Ford, I., Dubost-Brama, A., Lerebours, G., and Tavazzi, L. (2010) Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *Lancet* **376**, 875-885
3. DiFrancesco, D. (2010) The role of the funny current in pacemaker activity. *Circ. Res.* **106**, 434-446
4. Lakatta, E. G., Maltsev, V. A., and Vinogradova, T. M. (2010) A coupled SYSTEM of intracellular Ca<sup>2+</sup> clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ. Res.* **106**, 659-673
5. Boyett, M. R., Honjo, H., and Kodama, I. (2000) The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc. Res.* **47**, 658-687
6. Shibata, N., Inada, S., Mitsui, K., Honjo, H., Yamamoto, M., Niwa, R., Boyett, M. R., and Kodama, I. (2001) Pacemaker shift in the rabbit sinoatrial node in response to vagal nerve stimulation. *Exp. Physiol* **86**, 177-184
7. Mesirca, P., Torrente, A. G., and Mangoni, M. E. (2015) Functional role of voltage gated Ca(2+) channels in heart automaticity. *Front Physiol* **6**, 19
8. Torrente, A. G., Mesirca, P., Neco, P., Rizzetto, R., Dubel, S., Barrere, C., Sinegger-Brauns, M., Striessnig, J., Richard, S., Nargeot, J., Gomez, A. M., and Mangoni, M. E. (2016) L-type Cav1.3 channels regulate ryanodine receptor-dependent Ca<sup>2+</sup> release during sino-atrial node pacemaker activity. *Cardiovasc. Res.* **109**, 451-461
9. Priest, B. T. and McDermott, J. S. (2015) Cardiac ion channels. *Channels (Austin. )* **9**, 352-359
10. Lei, M., Jones, S. A., Liu, J., Lancaster, M. K., Fung, S. S., Dobrzynski, H., Camelliti, P., Maier, S. K., Noble, D., and Boyett, M. R. (2004) Requirement of neuronal- and cardiac-type sodium channels for murine sinoatrial node pacemaking. *J. Physiol* **559**, 835-848
11. Chambers, J. C., Zhao, J., Terracciano, C. M., Bezzina, C. R., Zhang, W., Kaba, R., Navaratnarajah, M., Lotlikar, A., Sehmi, J. S., Kooner, M. K., Deng, G., Siedlecka, U., Parasramka, S., El-Hamamsy, I., Wass, M. N., Dekker, L. R., de Jong, J. S., Sternberg, M. J., McKenna, W., Severs, N. J., de, S. R., Wilde, A. A., Anand, P., Yacoub, M., Scott, J., Elliott, P., Wood, J. N., and Kooner, J. S. (2010) Genetic variation in SCN10A influences cardiac conduction. *Nat. Genet.* **42**, 149-152
12. Mangoni, M. E., Couette, B., Bourinet, E., Platzer, J., Reimer, D., Striessnig, J., and



- Nargeot, J. (2003) Functional role of L-type Cav1.3 Ca<sup>2+</sup> channels in cardiac pacemaker activity. *Proc. Natl. Acad. Sci. U. S. A* **100**, 5543-5548
13. Mangoni, M. E., Traboulsie, A., Leoni, A. L., Couette, B., Marger, L., Le, Q. K., Kupfer, E., Cohen-Solal, A., Vilar, J., Shin, H. S., Escande, D., Charpentier, F., Nargeot, J., and Lory, P. (2006) Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels. *Circ. Res.* **98**, 1422-1430
  14. Vinogradova, T. M., Tagirova, S. S., and Lakatta, E. G. (2018) Unique Ca(2+)-Cycling Protein Abundance and Regulation Sustains Local Ca(2+) Releases and Spontaneous Firing of Rabbit Sinoatrial Node Cells. *Int. J. Mol. Sci.* **19**,
  15. Bogdanov, K. Y., Maltsev, V. A., Vinogradova, T. M., Lyashkov, A. E., Spurgeon, H. A., Stern, M. D., and Lakatta, E. G. (2006) Membrane potential fluctuations resulting from submembrane Ca<sup>2+</sup> releases in rabbit sinoatrial nodal cells impart an exponential phase to the late diastolic depolarization that controls their chronotropic state. *Circ. Res.* **99**, 979-987
  16. Sanders, L., Rakovic, S., Lowe, M., Mattick, P. A., and Terrar, D. A. (2006) Fundamental importance of Na<sup>+</sup>-Ca<sup>2+</sup> exchange for the pacemaking mechanism in guinea-pig sino-atrial node. *J. Physiol* **571**, 639-649
  17. Philipson, K. D. and Nishimoto, A. Y. (1980) Na<sup>+</sup>-Ca<sup>2+</sup> exchange is affected by membrane potential in cardiac sarcolemmal vesicles. *J. Biol. Chem.* **255**, 6880-6882
  18. Cheng, H., Lederer, W. J., and Cannell, M. B. (1993) Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* **262**, 740-744
  19. Bogdanov, K. Y., Vinogradova, T. M., and Lakatta, E. G. (2001) Sinoatrial nodal cell ryanodine receptor and Na(+)-Ca(2+) exchanger: molecular partners in pacemaker regulation. *Circ. Res.* **88**, 1254-1258
  20. Rigg, L., Heath, B. M., Cui, Y., and Terrar, D. A. (2000) Localisation and functional significance of ryanodine receptors during beta-adrenoceptor stimulation in the guinea-pig sino-atrial node. *Cardiovasc. Res.* **48**, 254-264
  21. Huser, J., Blatter, L. A., and Lipsius, S. L. (2000) Intracellular Ca<sup>2+</sup> release contributes to automaticity in cat atrial pacemaker cells. *J. Physiol* **524 Pt 2**, 415-422
  22. Sirenko, S., Yang, D., Li, Y., Lyashkov, A. E., Lukyanenko, Y. O., Lakatta, E. G., and Vinogradova, T. M. (2013) Ca(2+)-dependent phosphorylation of Ca(2+) cycling proteins generates robust rhythmic local Ca(2+) releases in cardiac pacemaker cells. *Sci. Signal.* **6**, ra6
  23. Vinogradova, T. M., Brochet, D. X., Sirenko, S., Li, Y., Spurgeon, H., and Lakatta, E. G. (2010) Sarcoplasmic reticulum Ca<sup>2+</sup> pumping kinetics regulates timing of local Ca<sup>2+</sup> releases and spontaneous beating rate of rabbit sinoatrial node pacemaker cells. *Circ. Res.* **107**, 767-775
  24. Andersson, K. B., Birkeland, J. A., Finsen, A. V., Louch, W. E., Sjaastad, I., Wang, Y.,

- Chen, J., Molkentin, J. D., Chien, K. R., Sejersted, O. M., and Christensen, G. (2009) Moderate heart dysfunction in mice with inducible cardiomyocyte-specific excision of the *Serca2* gene. *J. Mol. Cell Cardiol.* **47**, 180-187
25. Li, Y., Sirenko, S., Riordon, D. R., Yang, D., Spurgeon, H., Lakatta, E. G., and Vinogradova, T. M. (2016) CaMKII-dependent phosphorylation regulates basal cardiac pacemaker function via modulation of local Ca<sup>2+</sup> releases. *Am. J. Physiol Heart Circ. Physiol* **311**, H532-H544
  26. Groenke, S., Larson, E. D., Alber, S., Zhang, R., Lamp, S. T., Ren, X., Nakano, H., Jordan, M. C., Karagueuzian, H. S., Roos, K. P., Nakano, A., Proenza, C., Philipson, K. D., and Goldhaber, J. I. (2013) Complete atrial-specific knockout of sodium-calcium exchange eliminates sinoatrial node pacemaker activity. *PLoS. One.* **8**, e81633
  27. Zaritsky, J. J., Redell, J. B., Tempel, B. L., and Schwarz, T. L. (2001) The consequences of disrupting cardiac inwardly rectifying K(+) current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. *J. Physiol* **533**, 697-710
  28. Liu, G. X., Derst, C., Schlichtorl, G., Heinen, S., Seebohm, G., Bruggemann, A., Kummer, W., Veh, R. W., Daut, J., and Preisig-Muller, R. (2001) Comparison of cloned Kir2 channels with native inward rectifier K<sup>+</sup> channels from guinea-pig cardiomyocytes. *J. Physiol* **532**, 115-126
  29. Noma, A. and Trautwein, W. (1978) Relaxation of the ACh-induced potassium current in the rabbit sinoatrial node cell. *Pflügers Archiv* **377**, 193-200
  30. Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D. E. (1995) The G-protein-gated atrial K<sup>+</sup> channel IKACH is a heteromultimer of two inwardly rectifying K(+)-channel proteins. *Nature* **374**, 135-141
  31. Corey, S. and Clapham, D. E. (1998) Identification of native atrial G-protein-regulated inwardly rectifying K<sup>+</sup> (GIRK4) channel homomultimers. *Journal of Biological Chemistry* **273**, 27499-27504
  32. Fleischmann, B. K., Duan, Y., Fan, Y., Schoneberg, T., Ehlich, A., Lenka, N., Viatchenko-Karpinski, S., Pott, L., Hescheler, J., and Fakler, B. (2004) Differential subunit composition of the G protein-activated inward-rectifier potassium channel during cardiac development. *J. Clin. Invest* **114**, 994-1001
  33. Beckmann, C., Rinne, A., Littwitz, C., Mintert, E., Bosche, L. I., Kienitz, M. C., Pott, L., and Bender, K. (2008) G protein-activated (GIRK) current in rat ventricular myocytes is masked by constitutive inward rectifier current (I(K1)). *Cell Physiol Biochem.* **21**, 259-268
  34. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M., and Hille, B. (1985) GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature* **317**, 536-538
  35. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) The

beta gamma subunits of GTP-binding proteins activate the muscarinic K<sup>+</sup> channel in heart. *Nature* **325**, 321-326

36. Ford, C. E., Skiba, N. P., Bae, H., Daaka, Y., Reuveny, E., Shekter, L. R., Rosal, R., Weng, G., Yang, C.-S., Iyengar, R., Miller, R. J., Jan, L. Y., Lefkowitz, R. J., and Hamm, H. E. (1998) Molecular basis for interactions of G protein  $\beta\gamma$  subunits with effectors. *Science* **280**, 1271-1274
37. Slesinger, P. A., Reuveny, E., Jan, Y. N., and Jan, L. Y. (1995) Identification of structural elements involved in G protein gating of the GIRK1 potassium channel. *Neuron* **15**, 1145-1156
38. Whorton, M. R. and MacKinnon, R. (2011) Crystal structure of the mammalian GIRK2 K<sup>+</sup> channel and gating regulation by G proteins, PIP<sub>2</sub>, and sodium. *Cell* **147**, 199-208
39. Ho, I. H. M. and Murrell-Lagnado, R. D. (1999) Molecular determinants for sodium-dependent activation of G protein-gated K<sup>+</sup> channels. *Journal of Biological Chemistry* **274**, 8639-8648
40. Zhang, H., He, C., Yan, X., Mirshahi, T., and Logothetis, D. E. (1999) Activation of inwardly rectifying K<sup>+</sup> channels by distinct PtdIns(4,5)P<sub>2</sub> interactions. *Nat. Cell Biol.* **1**, 183-188
41. Leaney, J. L., Milligan, G., and Tinker, A. (2000) The G protein  $\alpha$  subunit has a key role in determining the specificity of coupling to, but not the activation of G protein-gated inwardly rectifying K<sup>+</sup> channels. *J. Biol. Chem.* **275**, 921-929
42. Riven, I., Iwanir, S., and Reuveny, E. (2006) GIRK channel activation involves a local rearrangement of a preformed G protein channel complex. *Neuron* **51**, 561-573
43. Clancy, S. M., Fowler, C. E., Finley, M., Suen, K. F., Arrabit, C., Berton, F., Kosaza, T., Casey, P. J., and Slesinger, P. A. (2005) Pertussis-toxin-sensitive Galpha subunits selectively bind to C-terminal domain of neuronal GIRK channels: evidence for a heterotrimeric G-protein-channel complex. *Mol. Cell Neurosci.* **28**, 375-389
44. Peleg, S., Varon, D., Ivanina, T., Dessauer, C. W., and Dascal, N. (2002) G( $\alpha$ )(i) controls the gating of the G protein-activated K(+) channel, GIRK. *Neuron* **33**, 87-99
45. Ivanina, T., Varon, D., Peleg, S., Rishal, I., Porozov, Y., Dessauer, C. W., Keren-Raifman, T., and Dascal, N. (2004) Galpha1 and Galpha3 differentially interact with, and regulate, the G protein-activated K<sup>+</sup> channel. *J. Biol. Chem.* **279**, 17260-17268
46. Nobles, M., Benians, A., and Tinker, A. (2005) Heterotrimeric G-proteins precouple with G-protein coupled receptors in living cells. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 18706-18711
47. Fisher, J. T., Vincent, S. G., Gomeza, J., Yamada, M., and Wess, J. (2004) Loss of vagally mediated bradycardia and bronchoconstriction in mice lacking M<sub>2</sub> or M<sub>3</sub> muscarinic acetylcholine receptors. *FASEB J.* **18**, 711-713
48. Wickman, K., Nemeč, J., Gendler, S. J., and Clapham, D. E. (1998) Abnormal heart

rate regulation in GIRK4 knockout mice. *Neuron* **20**, 103-114

49. Zuberi, Z., Birnbaumer, L., and Tinker, A. (2008) The role of inhibitory heterotrimeric G-proteins in the control of in-vivo heart rate dynamics. *Am. J. Physiol Regul. Integr. Comp Physiol* **295**, R1822-R1830
50. Gehrman, J., Meister, M., Maguire, C. T., Martins, D. C., Hammer, P. E., Neer, E. J., Berul, C. I., and Mende, U. (2002) Impaired parasympathetic heart rate control in mice with a reduction of functional G protein betagamma-subunits. *Am. J. Physiol Heart Circ. Physiol* **282**, H445-H456
51. DiFrancesco, D. and Tromba, C. (1987) Acetylcholine inhibits activation of the cardiac hyperpolarizing-activated current, *if*. *Pflugers Arch.* **410**, 139-142
52. Accili, E. A., Redaelli, G., and DiFrancesco, D. (1998) Two distinct pathways of muscarinic current responses in rabbit sino-atrial node myocytes. *Pflugers Arch.* **437**, 164-167
53. Boyett, M. R., Kodama, I., Honjo, H., Arai, A., Suzuki, R., and Toyama, J. (1995) Ionic basis of the chronotropic effect of acetylcholine on the rabbit sinoatrial node. *Cardiovasc. Res.* **29**, 867-878
54. Mesirca, P., Marger, L., Toyoda, F., Rizzetto, R., Audoubert, M., Dubel, S., Torrente, A. G., DiFrancesco, M. L., Muller, J. C., Leoni, A. L., Couette, B., Nargeot, J., Clapham, D. E., Wickman, K., and Mangoni, M. E. (2013) The G-protein-gated K<sup>+</sup> channel, IKACH, is required for regulation of pacemaker activity and recovery of resting heart rate after sympathetic stimulation. *J. Gen. Physiol* **142**, 113-126
55. Savonen, K. P., Lakka, T. A., Laukkanen, J. A., Halonen, P. M., Rauramaa, T. H., Salonen, J. T., and Rauramaa, R. (2006) Heart rate response during exercise test and cardiovascular mortality in middle-aged men. *Eur. Heart J.* **27**, 582-588
56. Yang, J., Huang, J., Maity, B., Gao, Z., Lorca, R. A., Gudmundsson, H., Li, J., Stewart, A., Swaminathan, P. D., Ibeawuchi, S. R., Shepherd, A., Chen, C. K., Kutschke, W., Mohler, P. J., Mohapatra, D. P., Anderson, M. E., and Fisher, R. A. (2010) RGS6, a modulator of parasympathetic activation in heart. *Circ. Res.* **107**, 1345-1349
57. Posokhova, E., Wydeven, N., Allen, K. L., Wickman, K., and Martemyanov, K. A. (2010) RGS6/Gss5 complex accelerates IKACH gating kinetics in atrial myocytes and modulates parasympathetic regulation of heart rate. *Circ. Res.* **107**, 1350-1354
58. Ramirez, J., Duijvenboden, S. V., Ntalla, I., Mifsud, B., Warren, H. R., Tzanis, E., Orini, M., Tinker, A., Lambiase, P. D., and Munroe, P. B. (2018) Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nat. Commun.* **9**, 1947
59. Mesirca, P., Alig, J., Torrente, A. G., Muller, J. C., Marger, L., Rollin, A., Marquilly, C., Vincent, A., Dubel, S., Bidaud, I., Fernandez, A., Seniuk, A., Engeland, B., Singh, J., Miquerol, L., Ehmke, H., Eschenhagen, T., Nargeot, J., Wickman, K., Isbrandt, D., and Mangoni, M. E. (2014) Cardiac arrhythmia induced by genetic silencing of 'funny' (f) channels is rescued by GIRK4 inactivation. *Nat. Commun.* **5**, 4664

60. Mesirca, P., Bidaud, I., Briec, F., Evain, S., Torrente, A. G., Le, Q. K., Leoni, A. L., Baudot, M., Marger, L., Chung You, C. A., Nargeot, J., Striessnig, J., Wickman, K., Charpentier, F., and Mangoni, M. E. (2016) G protein-gated IKACH channels as therapeutic targets for treatment of sick sinus syndrome and heart block. *Proc. Natl. Acad. Sci. U. S. A* **113**, E932-E941
61. Lei, M. and Brown, H. F. (1996) Two components of the delayed rectifier potassium current, IK, in rabbit sino-atrial node cells. *Exp. Physiol* **81**, 725-741
62. Smith, P. L., Baukrowitz, T., and Yellen, G. (1996) The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* **379**, 833-836
63. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* **269**, 92-95
64. Abbott, G. W., Sesti, F., Splawski, I., Buck, M. E., Lehmann, M. H., Timothy, K. W., Keating, M. T., and Goldstein, S. A. (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* **97**, 175-187
65. Verheijck, E. E., Wilders, R., and Bouman, L. N. (2002) Atrio-sinus interaction demonstrated by blockade of the rapid delayed rectifier current. *Circulation* **105**, 880-885
66. Clark, R. B., Mangoni, M. E., Lueger, A., Couette, B., Nargeot, J., and Giles, W. R. (2004) A rapidly activating delayed rectifier K<sup>+</sup> current regulates pacemaker activity in adult mouse sinoatrial node cells. *Am. J. Physiol Heart Circ. Physiol* **286**, H1757-H1766
67. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) K(V)LQT1 and IsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature* **384**, 78-80
68. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature* **384**, 80-83
69. Davies, M. P., Freeman, L. C., and Kass, R. S. (1996) Dual actions of the novel class III antiarrhythmic drug NE-10064 on delayed potassium channel currents in guinea pig ventricular and sinoatrial node cells. *J. Pharmacol. Exp. Ther.* **276**, 1149-1154
70. Lei, M., Brown, H. F., and Terrar, D. A. (2000) Modulation of delayed rectifier potassium current, iK, by isoprenaline in rabbit isolated pacemaker cells. *Exp. Physiol* **85**, 27-35
71. Bennett, P. B. and Begenisich, T. B. (1987) Catecholamines modulate the delayed rectifying potassium current (IK) in guinea pig ventricular myocytes. *Pflugers Arch.* **410**, 217-219
72. Marx, S. O., Kurokawa, J., Reiken, S., Motoike, H., D'Armiento, J., Marks, A. R., and Kass, R. S. (2002) Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*

73. Kurokawa, J., Chen, L., and Kass, R. S. (2003) Requirement of subunit expression for cAMP-mediated regulation of a heart potassium channel. *Proc. Natl. Acad. Sci. U. S. A* **100**, 2122-2127
74. Sanguinetti, M. C., Jurkiewicz, N. K., Scott, A., and Siegl, P. K. (1991) Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. *Circ. Res.* **68**, 77-84
75. Harmati, G., Banyasz, T., Barandi, L., Szentandrassy, N., Horvath, B., Szabo, G., Szentmiklosi, J. A., Szenasi, G., Nanasi, P. P., and Magyar, J. (2011) Effects of beta-adrenoceptor stimulation on delayed rectifier K(+) currents in canine ventricular cardiomyocytes. *Br. J. Pharmacol.* **162**, 890-896
76. Xie, Y., Ding, W. G., and Matsuura, H. (2015) Ca<sup>2+</sup>/calmodulin potentiates I Ks in sinoatrial node cells by activating Ca<sup>2+</sup>/calmodulin-dependent protein kinase II. *Pflugers Arch.* **467**, 241-251
77. Unudurthi, S. D., Wu, X., Qian, L., Amari, F., Onal, B., Li, N., Makara, M. A., Smith, S. A., Snyder, J., Fedorov, V. V., Coppola, V., Anderson, M. E., Mohler, P. J., and Hund, T. J. (2016) Two-Pore K<sup>+</sup> Channel TREK-1 Regulates Sinoatrial Node Membrane Excitability. *J. Am. Heart Assoc.* **5**, e002865
78. Bao, L., Kefaloyianni, E., Lader, J., Hong, M., Morley, G., Fishman, G. I., Sobie, E. A., and Coetzee, W. A. (2011) Unique properties of the ATP-sensitive K(+) channel in the mouse ventricular cardiac conduction system. *Circ. Arrhythm. Electrophysiol.* **4**, 926-935
79. Flagg, T. P., Kurata, H. T., Masia, R., Caputa, G., Magnuson, M. A., Lefer, D. J., Coetzee, W. A., and Nichols, C. G. (2008) Differential structure of atrial and ventricular KATP: atrial KATP channels require SUR1. *Circ. Res.* **103**, 1458-1465
80. Inagaki, N., Gonoi, T., Clement, J. P. 4., Namba, N., Inazawa, J., Gonzalez, G., Aguilar Bryan, L., Seino, S., and Bryan, J. (1995) Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270**, 1166-1170
81. Inagaki, N., Gonoi, T., Clement, J. P., Wang, C. Z., Aguilar Bryan, L., Bryan, J., and Seino, S. (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K<sup>+</sup> channels. *Neuron* **16**, 1011-1017
82. Aguilar Bryan, L., Nichols, C. G., Wechsler, S. W., Clement, J. P. 4., Boyd, A. E., Gonzalez, G., Herrera Sosa, H., Nguy, K., Bryan, J., and Nelson, D. A. (1995) Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* **268**, 423-426
83. Li, N., Wu, J. X., Ding, D., Cheng, J., Gao, N., and Chen, L. (2017) Structure of a Pancreatic ATP-Sensitive Potassium Channel. *Cell* **168**, 101-110
84. Martin, G. M., Yoshioka, C., Rex, E. A., Fay, J. F., Xie, Q., Whorton, M. R., Chen, J. Z., and Shyng, S. L. (2017) Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. *Elife.* **6**,

85. Lee, K. P. K., Chen, J., and MacKinnon, R. (2017) Molecular structure of human KATP in complex with ATP and ADP. *Elife*. **6**,
86. Noma, A. (1983) ATP-regulated K<sup>+</sup> channels in cardiac muscle. *Nature* **305**, 147-148
87. Tinker, A., Aziz, Q., and Thomas, A. (2014) The role of ATP-sensitive potassium channels in cellular function and protection in the cardiovascular system. *Br. J. Pharmacol.* **171**, 12-23
88. Zingman, L. V., Hodgson, D. M., Bast, P. H., Kane, G. C., Perez-Terzic, C., Gumina, R. J., Pucar, D., Bienengraeber, M., Dzeja, P. P., Miki, T., Seino, S., Alekseev, A. E., and Terzic, A. (2002) Kir6.2 is required for adaptation to stress. *Proc. Natl. Acad. Sci. U. S. A* **99**, 13278-13283
89. Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M., and Seino, S. (1995) Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J. Biol. Chem.* **270**, 5691-5694
90. Beech, D. J., Zhang, H., Nakao, K., and Bolton, T. B. (1993) K channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. *Br. J. Pharmacol.* **110**, 573-582
91. Cui, Y., Tran, S., Tinker, A., and Clapp, L. H. (2002) The molecular composition of K(ATP) channels in human pulmonary artery smooth muscle cells and their modulation by growth. *Am. J. Respir. Cell Mol. Biol.* **26**, 135-143
92. Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y., and Kurachi, Y. (1997) Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K<sup>+</sup> channel. *J. Physiol* **499** ( Pt 3), 715-720
93. Aziz, Q., Thomas, A. M., Gomes, J., Ang, R., Sones, W. R., Li, Y., Ng, K. E., Gee, L., and Tinker, A. (2014) The ATP-Sensitive Potassium Channel Subunit, Kir6.1, in Vascular Smooth Muscle Plays a Major Role in Blood Pressure Control. *Hypertension* **64**, 523-529
94. Fukuzaki, K., Sato, T., Miki, T., Seino, S., and Nakaya, H. (2008) Role of sarcolemmal ATP-sensitive K<sup>+</sup> channels in the regulation of sinoatrial node automaticity: an evaluation using Kir6.2-deficient mice. *J. Physiol* **586**, 2767-2778
95. Han, X., Light, P. E., Giles, W. R., and French, R. J. (1996) Identification and properties of an ATP-sensitive K<sup>+</sup> current in rabbit sino-atrial node pacemaker cells. *J. Physiol* **490** ( Pt 2), 337-350
96. Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H., and Seino, S. (2002) Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat. Med.* **8**, 466-472
97. Aziz, Q., Li, Y., Anderson, N., Ojake, L., Tsisanova, E., and Tinker, A. (2017) Molecular and functional characterization of the endothelial ATP-sensitive potassium channel. *J. Biol. Chem.* **292**, 17587-17597

98. Aziz, Q., Finlay, M., Montaigne, D., Ojake, L., Li, Y., Anderson, N., Ludwig, A., and Tinker, A. (2018) ATP-sensitive potassium channels in the sinoatrial node contribute to heart rate control and adaptation to hypoxia. *J. Biol. Chem.* **293**, 8912-8921
99. Hoesl, E., Stieber, J., Herrmann, S., Feil, S., Tybl, E., Hofmann, F., Feil, R., and Ludwig, A. (2008) Tamoxifen-inducible gene deletion in the cardiac conduction system. *J. Mol. Cell Cardiol.* **45**, 62-69
100. Boyett, M. R., Dobrzynski, H., Lancaster, M. K., Jones, S. A., Honjo, H., and Kodama, I. (2003) Sophisticated architecture is required for the sinoatrial node to perform its normal pacemaker function. *J. Cardiovasc. Electrophysiol.* **14**, 104-106
101. Giblin, J. P., Cui, Y., Clapp, L. H., and Tinker, A. (2002) Assembly limits the pharmacological complexity of ATP-sensitive potassium channels. *J. Biol. Chem.* **277**, 13717-13723
102. Glukhov, A. V., Flagg, T. P., Fedorov, V. V., Efimov, I. R., and Nichols, C. G. (2010) Differential K(ATP) channel pharmacology in intact mouse heart. *J. Mol. Cell Cardiol.* **48**, 152-160
103. Gerlach, A. C. and Antonio, B. M. (2015) Validation of ion channel targets. *Channels (Austin. )* **9**, 376-379
104. Vinogradova, T. M., Lyashkov, A. E., Zhu, W., Ruknudin, A. M., Sirenko, S., Yang, D., Deo, S., Barlow, M., Johnson, S., Caffrey, J. L., Zhou, Y. Y., Xiao, R. P., Cheng, H., Stern, M. D., Maltsev, V. A., and Lakatta, E. G. (2006) High basal protein kinase A-dependent phosphorylation drives rhythmic internal Ca<sup>2+</sup> store oscillations and spontaneous beating of cardiac pacemaker cells. *Circ. Res.* **98**, 505-514
105. Younes, A., Lyashkov, A. E., Graham, D., Sheydina, A., Volkova, M. V., Mitsak, M., Vinogradova, T. M., Lukyanenko, Y. O., Li, Y., Ruknudin, A. M., Boheler, K. R., van, E. J., and Lakatta, E. G. (2008) Ca(2+) -stimulated basal adenylyl cyclase activity localization in membrane lipid microdomains of cardiac sinoatrial nodal pacemaker cells. *J. Biol. Chem.* **283**, 14461-14468
106. Yaniv, Y., Ganesan, A., Yang, D., Ziman, B. D., Lyashkov, A. E., Levchenko, A., Zhang, J., and Lakatta, E. G. (2015) Real-time relationship between PKA biochemical signal network dynamics and increased action potential firing rate in heart pacemaker cells: Kinetics of PKA activation in heart pacemaker cells. *J. Mol. Cell Cardiol.* **86**, 168-178
107. Wilson, A. J., Jabr, R. I., and Clapp, L. H. (2000) Calcium modulation of vascular smooth muscle ATP-sensitive K(+) channels: role of protein phosphatase-2B. *Circ. Res.* **87**, 1019-1025
108. Orié, N. N., Thomas, A. M., Perrino, B. A., Tinker, A., and Clapp, L. H. (2009) Ca<sup>2+</sup>/calcineurin regulation of cloned vascular K ATP channels: crosstalk with the protein kinase A pathway. *Br. J. Pharmacol.* **157**, 554-564
109. Quayle, J. M., Nelson, M. T., and Standen, N. B. (1997) ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev.* **77**, 1165-1232



110. Quinn, K. V., Giblin, J. P., and Tinker, A. (2004) Multisite Phosphorylation Mechanism for Protein Kinase A Activation of the Smooth Muscle ATP-Sensitive K<sup>+</sup> Channel. *Circ. Res.* **94**, 1359-1366
111. Thery, C., Gosselin, B., Lekieffre, J., and Warembourg, H. (1977) Pathology of sinoatrial node. Correlations with electrocardiographic findings in 111 patients. *Am. Heart J.* **93**, 735-740
112. Evans, R. and Shaw, D. B. (1977) Pathological studies in sinoatrial disorder (sick sinus syndrome). *Br. Heart J.* **39**, 778-786
113. Choudhury, M., Boyett, M. R., and Morris, G. M. (2015) Biology of the Sinus Node and its Disease. *Arrhythm. Electrophysiol. Rev.* **4**, 28-34
114. Tellez, J. O., Mczewski, M., Yanni, J., Sutyagin, P., Mackiewicz, U., Atkinson, A., Inada, S., Beresewicz, A., Billeter, R., Dobrzynski, H., and Boyett, M. R. (2011) Ageing-dependent remodelling of ion channel and Ca<sup>2+</sup> clock genes underlying sinoatrial node pacemaking. *Exp. Physiol* **96**, 1163-1178
115. Allah, E. A., Tellez, J. O., Yanni, J., Nelson, T., Monfredi, O., Boyett, M. R., and Dobrzynski, H. (2011) Changes in the expression of ion channels, connexins and Ca<sup>2+</sup>-handling proteins in the sino-atrial node during postnatal development. *Exp. Physiol* **96**, 426-438
116. Opthof, T. (2000) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res.* **45**, 173-176
117. D'Souza, A., Bucchi, A., Johnsen, A. B., Logantha, S. J., Monfredi, O., Yanni, J., Prehar, S., Hart, G., Cartwright, E., Wisloff, U., Dobryznski, H., DiFrancesco, D., Morris, G. M., and Boyett, M. R. (2014) Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat. Commun.* **5**, 3775
118. Yanni, J., Tellez, J. O., Maczewski, M., Mackiewicz, U., Beresewicz, A., Billeter, R., Dobrzynski, H., and Boyett, M. R. (2011) Changes in ion channel gene expression underlying heart failure-induced sinoatrial node dysfunction. *Circ. Heart Fail.* **4**, 496-508
119. Bai, X., Wang, K., Yuan, Y., Li, Q., Dobrzynski, H., Boyett, M. R., Hancox, J. C., and Zhang, H. (2017) Mechanism underlying impaired cardiac pacemaking rhythm during ischemia: A simulation study. *Chaos.* **27**, 093934