

# It's not funny: How changes in $I_f$ limit maximum heart rate with aging

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Although many of us optimistically try to be “younger next year” (Crowley and Lodge, 2004), there is one inescapable fact: maximum heart rate declines over the decades regardless of fitness level. A parallel decrease in our so-called intrinsic heart rate, measured in the absence of sympathetic stimulation, indicates that these changes originate in the heart's pacemaker—the sinoatrial (SA) node (Larson et al., 2013). The problem goes well beyond the fitness goals of aging baby boomers, as pacemaking defects cause a host of cardiac dysrhythmias (Baruscotti et al., 2016). Thanks to ongoing studies from the laboratory of Cathy Proenza, including the current paper by Sharpe et al. in this issue of JGP, novel insights into the molecular basis of pacemaking may one day be translated into therapeutic treatments for arrhythmias and the effects of aging.

The investigators capitalize on a previous observation that maximal heart rate in mice, like humans, decreases with age and is accompanied by a parallel decrease in intrinsic or basal heart rate (Larson et al., 2013). The heart remains responsive to the sympathetic stimulation that increases heart rate, but the aging animal may not be able to fight or flee with the same verve. The source of this decline is, at least in part, a reduced intrinsic firing rate of the pacemaker cells in the SA node. And although other factors may contribute, the reduction in SA nodal firing rate is strongly correlated with changes in the ion channels that generate the “funny current” or  $I_f$  (Larson et al., 2013).  $I_f$ , activated by hyperpolarization while the heart is filling, allows sodium ions to enter the cell, causing the slow depolarization that triggers action potentials in the SA nodal cells and the subsequent spread of excitation throughout the heart. With more  $I_f$ , the SA node fires faster; with less  $I_f$ , as in aging cells, the firing rate is reduced.

$I_f$  is produced by HCN channels that gate open with hyperpolarizing voltages (hence “H”) and the binding of cyclic nucleotides (“CN”). The integration of these two signals is apparent in the conductance-voltage (G-V) plot describing the steady-state fraction of channels in the open (vs. closed) states as a function of voltage (Fig. 1). This S-shaped, or Boltzmann, relationship shifts along the voltage axis as cAMP levels change: when cAMP is elevated, a rightward (positive) shift indi-

cates that the channels can open more easily (at less negative voltages). The shifting of conductance along this axis is a key way that cAMP increases  $I_f$  and heart rate (DiFrancesco and Tortora, 1991; Wainger et al., 2001). In nodal cells of aging mice, the G-V for  $I_f$  is negatively shifted, contributing to the reduced SA node firing rate. Sympathetic stimulation increases both  $I_f$  and firing rate, but not to the levels observed in young animals (Larson et al., 2013).

The principal findings of this paper uncover new mechanisms underlying the age-related reduction in maximum heart rate, and there is both good and bad news. The bad news is that measures taken to increase endogenous levels of cellular cAMP or PKA, which also enhances  $I_f$  (Liao et al., 2010), do not restore  $I_f$  amplitude or SA pacemaker firing rate of aged cells to that of their junior counterparts. Moreover, the negative shift in the  $I_f$  G-V associated with aging persists even in excised macropatches, indicating that the shift is not associated with soluble factors (like cAMP) or labile modifications (like phosphorylation or PIP<sub>2</sub>). Instead, a stable change seems to render the  $I_f$  channels sluggish in aged cells. The good news is that  $I_f$  can be restored to its youthful levels, given ample cAMP applied through the patch pipette in whole-cell recordings or to excised macropatches. Importantly, pacemaker activity follows suit, firing at the same rates as cells from young animals when enough cAMP is present. Thus, there is a means by which the slowing of intrinsic and maximal heart rate can be thwarted, which is encouraging. How to exploit this finding for clinical applications will require more mechanistic insights, however, and so the work continues.

One main focus will be on identifying the factor or factors that inhibit  $I_f$  with age. Although  $I_f$  is thought to be composed primarily of HCN4  $\alpha$  subunits, other isoforms or  $\beta$  subunits can affect channel function (Li et al., 2015). Sharpe et al. (2017) favor the hypothesis that a protein like Trip8b, which modulates HCN1 channels in the brain, could be involved. Trip8b inhibits HCN1 by reducing its sensitivity to cAMP and the efficacy by which cAMP opens the channel. But in the presence of excess cAMP, the inhibitory effects of

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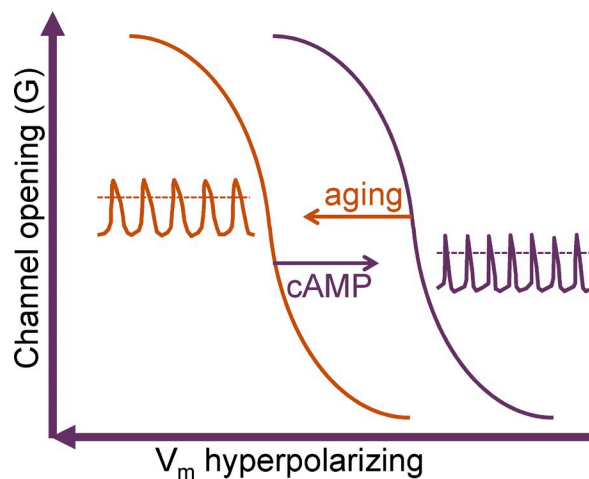


Figure 1. Channels underlying  $I_f$  “funny current” open with hyperpolarization (left along x axis). Aging causes a left (negative) shift in the  $G$ - $V_m$  relation (orange), with a corresponding reduction in  $I_f$  at a given voltage and slowing of pacemaker cell action potential frequency (and heart rate). cAMP applied exogenously can overcome the effects of aging on  $I_f$ , shifting the  $G$ - $V_m$  to more positive voltages characteristic of the young pacemaker cells with a corresponding increase in pacemaker cell firing rates. Adapted from Sharpe et al. (2017).

Trip8b can be overcome (Hu et al., 2013). This push-mi-pullyu effect is achieved by mutual antagonism of Trip8b and cAMP actions via allosteric mechanisms at distinct sites on the channel (Santoro et al., 2011; Saponaro et al., 2014; DeBerg et al., 2015, 2016). The effects of Trip8b on HCN1 channels are strikingly similar to those of aging on  $I_f$ .

Because Trip8b is not found in the heart (Santoro et al., 2004), the findings here warrant a multipronged approach involving bioinformatics to find proteins related to Trip8b, experiments to see whether they are expressed in the SA node, and assays for their effects on HCN4 gating. Alternatively, for one with the stomach to take on a yeast two-hybrid screen, it may be necessary to follow in the pioneering footsteps of Santoro et al. (2004) who first identified Trip8b and look for its functional counterpart in the heart. Now that the structural basis for Trip8b interaction with HCN1 has been resolved (Saponaro et al., 2014; DeBerg et al., 2015), a targeted approach using the corresponding regions on HCN4 as bait may prove fruitful and perhaps more direct than the original efforts. Human genetics may also advance the cause, following the emergence of *HCN4* as a potential target for sinus node disease, atrial fibrillation, and other arrhythmias (Baruscotti et al., 2016). Other genes encoding interacting proteins that modulate HCN4 function may similarly be identified as disease targets. There is reason to be optimistic, as Sharpe et al. (2017) discovered a factor with TRIP8b-like properties but specific for HCN4. The as-yet-unidentified factor was present in Chinese hamster ovary (CHO) cells but not human embry-

onic kidney (HEK) cells (Liao et al., 2012). If luck follows serendipity, this factor may be identified in the near future.

Like most good papers, more questions are raised by Sharpe et al. (2017) than are answered, and limitations exist. The authors acknowledge the differences between the whirring mouse models and the ambling human heart, but it should be possible to replicate the work in SA nodal cells from a large mammalian preparation (Gao et al., 2010) or in SA nodal-like cells derived from human induced pluripotent stem cells (Ben-Ari et al., 2014; Jung et al., 2014). Given the remarkable parallels in SA nodal function established to date, it seems likely that more commonalities await discovery. In addition, our understanding of the mechanism by which cyclic nucleotide binding is communicated to the gating machinery is steadily evolving, with structural insights into the conformations of cyclic nucleotide-binding domains (Zagotta et al., 2003; Clayton et al., 2004; Saponaro et al., 2014) and single-molecule studies that dynamically monitor the transitions evoked by ligand binding (Goldschen-Ohm et al., 2016). In the meantime, whether it's on the hamster wheel or the treadmill, we must face the limitations of aging, knowing that in our hearts, at least, lies the potential to reverse the trend.

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