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Viewpoint

Keeping the clocks ticking as we age: changes in sinoatrial node gene expression and function in the ageing heart

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The function of the sinoatrial node (SAN; the natural pacemaker of the heart) declines with age, leading to a condition called sick sinus syndrome, which is associated with a variety of cardiac arrhythmias or conduction disturbances (Dobrzynski *et al.* 2007). Sick sinus syndrome accounts for more than 50% of pacemaker implantations in people over 60 years of age. The decline in SAN function with age may occur as a result of structural changes (collagen deposition), alterations in ion channels (expression and/or function may be perturbed) and impairments in cell-to-cell communication (altered gap junction function). In this issue of *Experimental Physiology*, Tellez *et al.* (2011) have quantitatively characterized the expression of 86 mRNA transcripts (including ion channels, transporters, connexins and receptors) in the SAN and atrial myocardium from young (3 months) and aged (25 months) rats. Their data illustrate the enormous complexity of the ageing process in the heart.

The SAN is able to generate the heart beat rhythmically owing to the unique electrophysiological properties of its specialized pacemaker cells, which generate spontaneous action potentials (APs) that are distinct from the surrounding atrial myocardium (Mangoni & Nargeot, 2008). Critical to pacemaker activity, the SAN myocyte gradually depolarizes during phase 4 of the AP waveform until the threshold for the next AP is reached. This gradual depolarization is called the diastolic depolarization and is the fundamental feature of the SAN myocyte that enables pacemaker activity. Several ionic mechanisms have been shown to play a role in the generation and regulation of the diastolic depolarization (Mangoni & Nargeot, 2008). These include, among others, the hyperpolarization-activated current (I_f) carried by HCN channels, T- and L-type Ca^{2+} currents ($I_{Ca,T}$ and $I_{Ca,L}$), delayed rectifier K^+ currents (I_K) and an inward Na^+–Ca^{2+} exchange current (I_{NCX}) driven by the release of Ca^{2+} from the sarcoplasmic reticulum (SR). The terms 'membrane clock' and 'Ca^{2+} clock' have been coined to describe, respectively, the contributions of ion channels in the plasma membrane and Ca^{2+} release from the SR to pacemaker activity (Lakatta *et al.* 2010). The relative importance of some of these mechanisms, particularly I_f and I_{NCX} due to SR Ca^{2+} release, continues to be debated (Mangoni & Nargeot, 2008; Lakatta *et al.* 2010).

In the aged heart, SAN dysfunction is characterized by reductions in the frequency of spontaneous AP firing and the rate of diastolic depolarization, as well as an increase in AP duration in SAN myocytes (Dobrzynski *et al.* 2007). In the whole heart, this manifests as an increase in SAN recovery time and a reduction in intrinsic heart rate (de Marneffe *et al.* 1993). These electrophysiological changes were confirmed by Tellez *et al.* (2011). The extensive gene expression studies demonstrate that, of the 86 transcripts studied, 24% changed significantly with age in the SAN, while 27% changed significantly with age in the atrial myocardium. Notable changes that occurred in the SAN that may contribute to a reduction in intrinsic heart rate include reductions in the expression of RYR2, HCN1 and connexin 30.2 (Cx30.2). The age-related prolongation of AP duration is suggested to be related to increases in the expression of $Na_V1.5$ and $Ca_V1.2$, as well as a reduction in $K_V1.5$. The contribution of RYR2 to age-related SAN dysfunction was investigated further. Specifically, RYR2 protein expression was assessed by immunohistochemistry and, like mRNA levels, was shown to be significantly reduced in the SAN of aged rats. Functionally, application of ryanodine, which depletes the SR of Ca^{2+} and thus removes the contribution of SR Ca^{2+} release to pacemaker function, reduced intrinsic heart rate in young rats by ~40%. Ryanodine was without effect in the aged animals, which already displayed a lower intrinsic heart rate compared with the young animals. Based on these data, it is suggested that a reduction in the contribution of SR Ca^{2+} release to pacemaker function in the SAN (i.e. Ca^{2+} clock mechanisms) is a major mediator of SAN dysfunction and impaired heart rate regulation with age.

Tellez *et al.* (2011) have provided an extensive characterization of the changes in gene expression that occur in the SAN (and atria) as a function of age. There were significant changes (both increases and decreases) in the expression of a number of transcripts thought to play a role in maintaining or regulating SAN function. As such, this study raises many interesting and important questions. Importantly, and as acknowledged by the authors, mRNA levels do not necessarily correlate with protein levels and/or the functional roles of proteins. Only RYR2 was studied beyond the mRNA level; therefore, the role(s) of the many other transcripts that changed with age are unclear at this time. For example, RYR2 is only one component of the Ca^{2+}-handling system in SAN myocytes. The sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) and its regulatory protein phospholamban are responsible for Ca^{2+} reuptake by the SR, thereby setting SR Ca^{2+} load, which is an important determinant of Ca^{2+} release via RYR2. Expression of SERCA was increased in aged rats, which may partly counteract the effect of a reduction in RYR2 expression and function. Likewise, expression of the inositol 1,4,5-triphosphate receptor (IP_3R3) was increased in aged SAN, which could further promote SR Ca^{2+} loading if protein expression and function are also enhanced. The expression of phospholamban was not studied and remains to be determined. Although RYR2 appears to contribute prominently to SAN dysfunction in old rats, changes in other transcripts, such as HCN1 and Cx30.2, which were also reduced, could make equally important contributions. In contrast, based on studies showing a reduction in SAN pacemaker function in mice with impaired Na^+ channels (reviewed by Mangoni & Nargeot, 2008), the increase in $Na_V1.5$ mRNA in the SAN of aged rats would predict an increase in heart rate if this were to translate to more Na^+ channels

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in the membrane. Only functional studies will be able to address how these and other changes in mRNA expression contribute to the overall reduction in intrinsic heart rate that occurs with age. In summary, Tellez *et al.* (2011) have generated a fascinating data set that provides important insight into the complex changes that occur in the SAN during ageing. This study should serve as a launching pad for a number of functional studies aimed at determining the mechanism(s) responsible for SAN dysfunction in the aged heart and ultimately how we can keep our clocks ticking as we get older.

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