Involvement of calcium in pacemaker firing

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Pacemaker firing in the heart was initially considered to result from the coupled activity of a series of voltage-sensitive channels (voltage clock). The discovery that ryanodine, a blocker of sarcoplasmic reticulum (SR) Ca^{2+} release, slowed or stopped pacemaker firing first suggested that intracellular Ca^{2+} contributed to the process (calcium clock). Ju & Allen (1998) suggested that Ca^{2+} released from the SR was extruded from the cell on the Na/Ca exchanger and that this process generated an inward current that contributed to the pacemaker potential primarily during early diastole. Lakatta and his group (Vinogradova *et al.*, 2004) focused on Ca^{2+} sparks, which occur only when the Ca^{2+} content of the SR is high and therefore in late in diastole, and showed that the close localization of Ca^{2+} release sites and Na/Ca exchangers led to a component of inward current late in diastole.

We have recently discovered two other Ca^{2+} pathways in pacemaker cells with potential roles in Ca^{2+} regulation and therefore firing rate. Pacemaker cells, like most cell types, possess a store-operated Ca^{2+} current (Ju *et al.*, 2007) which provides Ca^{2+} influx, and presumably an inward current, whenever the store is sufficiently depleted of Ca^{2+}. Whether this current is turned on briefly at the end of each systole or whether it is background current which reflects the time-averaged level of Ca^{2+} in the SR is currently unknown. When the store is depleted and the store-operated Ca^{2+} current is activated, the effect on firing rate is likely to be complex because simultaneously the SR Ca^{2+} release will be reduced and therefore Na/Ca related current small whereas the store-operated Ca^{2+} current will be turned on. Thus the net effect on firing rate is not intuitively obvious.

A second novel Ca^{2+} pathway in pacemaker cells is provided by IP3 receptors which we recently showed to be present in the SR of pacemaker cells (Ju *et al.*, 2011). The main Ca^{2+} release channel in cardiac SR is the ryanodine receptor (RyR2) which is Ca^{2+}-sensitive and activated by the Ca^{2+} influx through the L-type Ca^{2+} channels in the surface membrane. In contrast IP3 receptors (IP3 R2) are ~50 fold less frequent and activated by IP3 but not by Ca^{2+}. Nevertheless we have shown that IP3 agonist and antagonists, increase or decrease the firing rate, respectively, and that these effects are absent in IP3 R2 KO mice (Ju *et al.*, 2011).

One challenge for these novel Ca^{2+} pathways is to determine whether they make a contribution to the normal firing rate and, if so, under what circumstances. The pharmacological and genetic tools for analysing these contributions are imperfect, so an alternative is to use mathematical modelling. We have added a store-operated Ca^{2+} channel (Allen *et al.*, 2012) to an existing pacemaker model (Imtiaz *et al.*, 2010) and explored the effects on firing rate. We have also added IP3 receptors to the SR in the model and have demonstrated how their presence modulates Ca^{2+} handling and pacemaker firing rate.

Allen DG, Ju YK, Liu J, & Imtiaz M (2012). In Store-operated Ca^{2+} entry pathways, eds. Groschner K, Graier WF, & Romanin C, Springer, Wien, Austria.

Imtiaz MS, von der Weid PY, Laver DR, & Van Helden DF (2010). *Journal of Molecular and Cellular Cardiology* **49**, 412-426.

Ju YK & Allen DG (1998). *Journal of Physiology* **508**, 153-166.

Ju YK, Chu Y, Chaulet H, Lai D, Gervasio OL, Graham RM, Cannell MB, & Allen DG (2007). *Circulation Resesearch* **100**, 1605-1614.

Ju YK, Liu J, Lee BH, Lai D, Woodcock EA, Lei M, Cannell MB, & Allen DG (2011). *Circulation Resesearch* **109**, 848-857.

Vinogradova TM, Zhou YY, Maltsev V, Lyashkov A, Stern M, & Lakatta EG (2004). *Circulation Resesearch* **94**, 802-809.

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