Beating to time: calcium clocks, voltage clocks, and cardiac pacemaker activity

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THE HEARTBEAT ARISES in the sinoatrial node (SAN) and then spreads throughout the heart. SAN function is therefore essential for normal cardiac physiology. The function of the SAN decreases with age, and this has been correlated with changes of ion channel expression (12). It is therefore of great importance to understand the mechanisms responsible for normal pacemaker activity in the SAN. Writing an Editorial on this subject would have been much easier in the 1980s. By then, it had been shown that a hyperpolarization-activated current, Iₘ, underlies the pacemaker depolarization. This current (often referred to as the “funny” current because, unlike the majority of voltage-sensitive currents, it is activated by hyperpolarization rather than depolarization) turns on at the end of the action potential and then depolarizes the membrane to a level where the Ca²⁺ current (I₉) activates to initiate the action potential. See Refs. 7 and 19 for reviews. The major role of Iₘ has been reinforced by the fact that drugs such as ivabradine targeted to block Iₘ (2) slow heart rate (8) and also mutations in the Iₘ channel are associated with slowed heart rate (21).

An early suggestion that the control of pacemaker activity might not be restricted to sarcolemmal channels and, specifically, that it might involve intracellular calcium regulation came from the observation that application of ryanodine slowed pacemaker activity in secondary (atrial) pacemaker tissue (24). Subsequent work in this tissue showed the involvement of the electrogenic Na⁺-Ca²⁺ exchanger (NCX) in pacemaker activity and also raised the possibility that increased Ca release and hence NCX current could play a role in the positive chronotropic effect of β-adrenergic stimulation (29). SAN pacemaker activity was also slowed by interfering with sarcoplasmic reticulum (SR) Ca release (ryanodine) or uptake (cyclopiazonic acid; CPA) (22). Work on toad pacemaker cells showed that ryanodine abolished pacemaker activity. In that study, removal of sodium also abolished spontaneous activity leading to the suggestion that Ca release from the SR-activated NCX thereby depolarizing the cell (13). The final piece of evidence linking calcium release to pacemaker activity was provided by Huser et al. (11) who demonstrated that calcium sparks occurred during the pacemaker depolarization (again in latent pacemaker cells) and suggested that these could activate NCX and thereby depolarize the cell. The next year, this hypothesis was shown to also apply to the SAN itself with the demonstration that local Ca release produced pacemaker depolarization (1). Spontaneous activity of immature cardiomyocytes from embryonic stem cells also exhibits a strong dependence on intracellular Ca²⁺ signaling mechanisms (14). Thus, from an ontogenetic point of view, this “primordial” property might be seen as a developmental step that is retained in SAN in the adult heart.

This pacemaker activity produced by calcium release from the SR-activating NCX is reminiscent of the abnormal pacemaker activity long known to be responsible for triggered arrhythmias (see Ref. 26 for review). As initially observed during digitalis intoxication (9, 23), calcium overload results in delayed afterdepolarizations (DADs). These originate from a transient inward current (now identified as NCX current; Ref. 20) associated with an increase of [Ca²⁺]c (15). Under normal circumstances, calcium release from the SR is triggered by the sarcolemmal influx on the L-type current activating the ryanodine receptor (RyR). However, if the SR Ca content exceeds a threshold level (6), then Ca release can occur spontaneously and propagates through the cell as a wave. If this wave occurs in diastole, the resulting NCX current may depolarize the membrane sufficiently to result in a spontaneous (ectopic) action potential. It appears, therefore, that the same mechanism that produces some triggered arrhythmias is responsible for normal pacemaker activity. This suggests that the SAN must exist normally in a state of calcium overload. This has been shown to be due to a high basal level of PKA activity in the SAN (28).

The above highlights the fact that SAN pacemaker activity depends on at least two mechanisms: 1) the activity of voltage-dependent sarcolemmal currents (Iₘ, I₉, etc.); and 2) time-dependent release of Ca from the SR-activating depolarizing NCX current. Although it appears that either mechanism alone would be capable of producing pacemaker activity, the literature contains much discussion about the relative importance of the two mechanisms. A further debate has arisen around their individual (or mutual) relevance in mediating the positive/negative chronotropic effect of neurotransmitters (3, 27). In this context, an element of complexity resides in the coupling between the two mechanisms: the sarcolemmal Ca current will refill the SR with calcium, and, reciprocally, the Ca released from the SR will activate NCX, change membrane potential, and thereby affect the sarcolemmal mechanisms. The combination of these two mechanisms is therefore complicated and defies intuition. However, a deeper comprehension of SAN activity and the development of reliable mathematical models may help to predict or interpret the effects of diseases [e.g., heart failure (25)] or drugs [e.g., lithium (10)].

In their article Maltsev and Lakatta (18) develop a model of both mechanisms that they describe as calcium and voltage “clocks.” The possibility to model SAN activity has provided some interesting ideas that can be tested. The authors have dissected out the effects of interfering with either the calcium clock (using ryanodine to block the RyR) or the voltage clock (using cesium to block Iₘ). Their modeling predicts that ryan-
odine will abolish rhythmic activity. This result is in line with experimental work from their own laboratory showing that high concentrations of ryanodine (30 μM) abolish activity in 83% of rabbit SAN cells (17). It is, however, surprising that, even at these high concentrations, the effect takes 15 min to develop, and it is therefore possible that effects secondary to changes of [Ca^{2+}] are also involved. This is also suggested by changes in maximum diastolic potential and take off potentials (i.e., the voltage level at which diastolic depolarization turns into the action potential upstroke) both in silico (18) and in vitro (3). Finally, it should be noted that (also in the rabbit SAN) other workers found ryanodine to have much smaller effects on cycle length (16). At a concentration between 2 and 30 μM, the effects of ryanodine depended on the size of the cell with small cells being unaffected and rate being slowed by 27% in larger cells; the original study of Rigg and Terrar (22) also reported smaller effects of ryanodine. As regards the effects of cesium, the present model predicts only a 5% slowing of rate by completely inhibiting \(I_{f}\). Whereas this very small effect is consistent with the authors’ own experimental work, it differs from the larger effects of blocking \(I_{f}\) found by others (4, 5). In conclusion, the current model is a very useful tool, and it will be of great interest in the future to see how it can account for the diversity of experimental findings found by different groups.

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REFERENCES