Letter to the editor: “Validating the requirement for beat-to-beat coupling of the Ca$^{2+}$ clock and M clock in pacemaker cell normal automaticity”

Victor A. Maltsev, Tatiana M. Vinogradova, Michael D. Stern, and Edward G. Lakatta

Laboratory of Cardiovascular Science, Gerontology Research Center, Baltimore, Maryland

TO THE EDITOR: A novel, general concept of a coupled-clock pacemaker cell system of surface membrane electrogenic proteins that function as a voltage oscillator (M clock) and of intracellular proteins that effect diastolic submembrane Ca$^{2+}$ oscillations (Ca$^{2+}$ clock) has been recently put forth (5). The essence of the “yin-yang”-type dynamic coupling of the clocks is based on, in part, several lines of robust experimental data. First, an abrupt disabling of the Na$^+/Ca^{2+}$ exchanger function by “spritzing” Na$^+$ free medium during diastolic depolarization (DD) acutely retards the normal DD sinoatrial node cells (SANCs) (1, 10). Second, for sarcoplasmic reticulum (SR) Ca$^{2+}$ load transitions, after removal of voltage clamp at the maximum diastolic potential that depletes SR Ca$^{2+}$ load, the beat-to-beat reduction in cycle length is predicted by the beat-to-beat growth of local submembrane Ca$^{2+}$ releases (LCRs) that activate an inward Na$^+/Ca^{2+}$ exchanger current during DD (11). Third, stochastic beat-to-beat variations in LCR emergence during DD within a given SANC predict the cell’s intrinsic cycle length variability (7). Both Ca$^{2+}$ release and sarcotubular myofilament channels are involved in mechanisms of intrinsic beating variability in cardiac cell cultures (8). Fourth, for the existence of Ca$^{2+}$ clock, rhythmic LCRs persist under voltage clamp and in chemically skinned SANCs when cell Ca$^{2+}$ is sustained.

In the January 2011 issue of the American Journal of Physiology-Heart and Circulatory Physiology, Himeno et al. (3) report patch-clamp experiments and numerical modeling in guinea pig SANCs that seem to refute the coupled-clock hypothesis. But some issues, not considered by the authors, might be important for the interpretation of their results.

Result 1

The spontaneous action potential (AP) rate changed little when BAPTA, a Ca$^{2+}$ chelator, was acutely infused via a patch pipette into SANCs.

Our interpretation. The unexpected BAPTA effect, however, can be explained on the basis of a small, artificial patch seal leak current, a well-recognized artifact in whole cell patch-clamp experiments that occurs when the membrane patch is ruptured. The occurrence of such leak currents immediately after patch rupture in the experiments of Himeno et al. (3) is evidenced by a clearly notable acute depolarization of maximum diastolic potential [shown by arrow in Fig. 5A,a in Himeno et al. (3)]. The depolarization is accompanied by an acute cell contracture (incomplete relaxation), likely resulting from Ca$^{2+}$ nearly and instantaneously entering the cell from the bath through the patch seal ahead of BAPTA diffusion from the pipette. The artificial leak currents pose a serious problem during measurements of spontaneous AP firing, because the DD is driven by a net current of a few pico-amperes (2). In other words, small leak currents associated with seal electrical conductance likely shift the current balance of tiny DD currents, artificially increase the DD rate, and offset the true BAPTA effect that would, in the absence of the artifact, suppress the DD and prolong the cycle length.

Result 2

SANC automaticity persists upon acute intracellular BAPTA application in simulations of a numerical M-clock model of SANCs, whereas a recent coupled-clock model [Maltsev-Lakatta (ML) model (6)] predicts pacemaker failure. Since failure was not observed experimentally, the authors concluded that the ML model is inaccurate [Fig. 4 vs. Fig. 5A,a in Himeno et al. (3)].

Our interpretation. With an introduction of a small leak current (~8 pA during DD through a 6-GΩ seal resistance) simulating the experimental conditions of Himeno et al. (3), the coupled-clock ML model does not fail but continues to generate spontaneous APs when its Ca$^{2+}$ clock is acutely disabled (SR Ca$^{2+}$ pumping rate set to zero; see Fig. 1).

Result 3

While intracellular BAPTA application does not acutely affect SANC automaticity, spontaneous activity is disrupted after several minutes. The authors explain this result based on the long-term effects of Ca$^{2+}$ on CaMII and/or the Ca$^{2+}$-stimulated adenylyl cyclase [See DISCUSSION in Himeno et al. (3)].

Our interpretation. The experimental configuration of Himeno et al. (3) does not permit the study of long-term effects, because upon membrane rupture in transition from perforated patch clamp to whole cell configuration, the perforating agent (amphotericin B) quickly diffuses from patch pipette into cytosol and perforates the whole cell membrane, resulting in large artificial leak currents and excitability loss.

Result 4

During the first 150 s of cell exposure to a mixture of ryanodine and thapsigargin, the spontaneous AP firing rate is slightly increased but cell contraction is notably decreased [Fig. 7 in Himeno et al. (3)].

Our interpretation. During such a short time, the drugs’ mixture incompletely disables the SR’s Ca$^{2+}$ pumping-release function. Previous studies demonstrated that the effect of thapsigargin to disable SR Ca$^{2+}$ load requires a longer time, up to 20–30 min (4). Moreover, it is also well known that ryanodine locks Ca$^{2+}$ release channels [ryanodine receptors (RyRs)] in a subconducting, open state. Therefore, before SR Ca$^{2+}$ depletion, Ca$^{2+}$ flux from the SR via RyRs is initially increased by ryanodine, resulting in an acute increase in AP firing rate demonstrated as early as in 1989 (9). This unnatural
Ryanodine-subconductance open state occurs shortly after ryanodine binding to RyR and can also explain the reduction in contraction amplitude in the experiments of Himeno et al. (3) since it concomitantly impairs the normal process of the excitation-contraction coupling (via Ca\(^{2+}\)-induced Ca\(^{2+}\) release, normally effectively triggered by L-type Ca\(^{2+}\) current).

**Summary**

The issues addressed by Himeno et al. (3) are crucial to test the hypothesis of beat-to-beat regulation of a coupled-clock pacemaker system. In our opinion, however, the methods they have chosen to test these issues have severe shortcomings and therefore preclude rigorous interpretations. Additional experiments employing techniques that do not suffer from such shortcomings are required in this case, for example, simultaneous measurement of intracellular Ca\(^{2+}\) concentration and APs by perforated patch clamp during the entire duration of exposure of SANCs to ryanodine and the photolysis of a caged Ca\(^{2+}\) buffer.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**