

BIOCHEMISTRY, BIOPHYSICS,
AND MOLECULAR BIOLOGY

Study of the Effect of Acetylcholine on Intracellular Homeostasis of True Pacemaker Cells of Rabbit Sinus Node Using Computer Simulation

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Acetylcholine, a neurotransmitter secreted by postganglionic parasympathetic termini, plays a key role in the regulation of spontaneous activity and excitation propagation in the sinus node of mammals in normalcy and pathology. In previous works, we studied the effect of acetylcholine on electric activity of sinus-node cells [1, 2]. In this study, we investigated the effect of acetylcholine on intracellular ion homeostasis. We found that the characteristic time of the onset of homeostasis ($T_{1/2}$) was 34 s for sodium and potassium ions and 1 s for calcium ions. A considerable difference in values is determined by the functioning of sarcoplasmic reticulum (SR).

Conditions of numerical experiments. The descriptions of the model of the electrical activity of sinus-node cell membranes, the effect of acetylcholine, and the methods of numerical integration used in this study were described in [1–4]. When simulating the slow dynamics of calcium, the Ca-ATPase current was also taken into consideration [5]. To simulate intracellular homeostasis, we took into account changes in the concentration of Na^+ , K^+ , and Ca^{2+} in the cell. The balance of these ions was determined by the respective incoming and outgoing membrane currents (Fig. 1). To determine Ca^{2+} balance, the function of SR (specifically, Ca^{2+} uptake by SERCA2 pump, accumulation of Ca^{2+} in network SR (NSR), its diffusion into terminal cisterns (JSR), and subsequent release of Ca^{2+} through the ryanodine receptors), as well as Ca^{2+} binding by troponin, calmodulin, and calsequestrin, was taken into account. The model of functioning SR in rabbit sinus-node cells has been described in more detail in [5].

Results. The results of a short-term (10-s) exposure to acetylcholine are shown in Fig. 2. The concentration of acetylcholine equal to 25 nM was reached within 5–15 s, which led to a slight decrease in the amplitude of oscillations and deceleration of the rhythm. Acetylcho-

line had opposite effects on the concentrations of different ions in the cell: Na^+ concentration gradually increased, K^+ concentration gradually decreased, and Ca^{2+} concentration rapidly adapted to new conditions. Apparently, Na^+ and K^+ , in contrast to Ca^{2+} , do not reach steady-state level within the time interval specified.

The time course of Ca^{2+} in the sarcoplasm and SR is shown in Fig. 3. Due to a special regulatory system in SR, the concentration of Ca^{2+} is rapidly adapted to new steady-state values determined by the effect of acetylcholine. Note that the lowest Ca^{2+} concentration (Ca_i , 0.2–0.4 μM) was detected in the sarcoplasm; in the diadic space (in complexes between ryanodine receptors and L-type calcium channels), Ca^{2+} concentration was higher (Ca_{sub} , 0.4–0.8 μM). In SR, Ca^{2+} concentration reached millimolar values (Ca_{up} , 1 mM); in terminal cisterns of SR, Ca^{2+} concentration was lower by an order of magnitude (Ca_{rel} , 0.02–0.1 mM). Interestingly, acetylcholine caused nonuniform changes in Ca^{2+} concentration within SR. In NSR, exposure to acetylcholine caused a slight decrease in Ca_{up} ; however, in terminal cisterns of JSR, Ca_{rel} was much greater than in the control (Fig. 3). Such a time course can be accounted for by the complex nonlinear function of SR.

Because the process of steady-state onset for Na^+ and K^+ is very slow, more long-term measurements are required. Figure 4 shows the results of simulation of the effect of 25 nM acetylcholine, which was introduced into the system within 5–400 s. It can be seen that the characteristic time of homeostasis onset ($T_{1/2}$) was equal to 34 s for Na^+ and K^+ and approximately 1 s for Ca^{2+} .

Discussion. The effect of acetylcholine on electrophysiological parameters of sinus-node cells is the subject of intensive long-term studies. Little is known on the effect of acetylcholine on the intracellular ion homeostasis, because precise methods of measurements of concentrations of ions in living cells are absent thus far. The use of computer simulation remains the only available method to study this effect. Computer simulation allowed us to estimate the value and

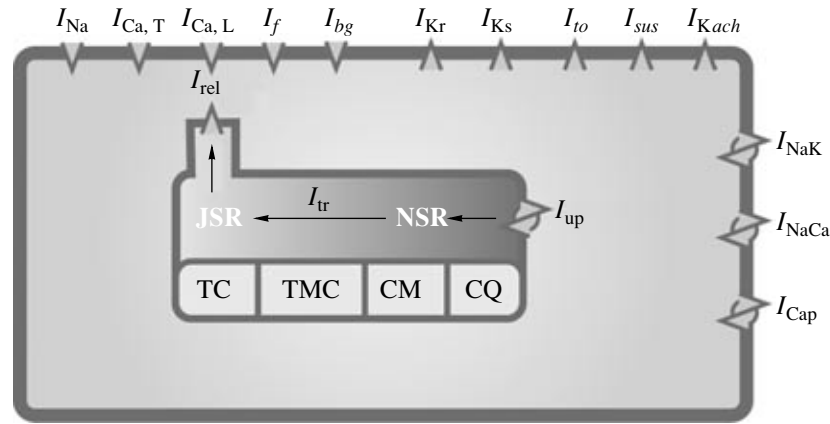


Fig. 1. Scheme of membrane and intracellular currents in the model of rabbit sinus-node cells. Designations: I_{Na} , sodium current; $I_{Ca,T}$ and $I_{Ca,L}$, T- and L-type calcium currents; I_f , hyperpolarization-activated current; I_{bg} , background current; I_{Kr} and I_{Ks} , rapid and slow potassium currents of delayed rectification; I_{to} and I_{sus} , components of 4-AP-sensitive current; I_{Kach} , ACh-activated potassium current; I_{NaK} , Na-K-pump; I_{NaCa} , Na-Ca exchanger; I_{Cap} , Ca-pump; I_{rel} , ryanodine-receptor calcium current; I_{up} , SERCA-pump; I_{tr} , calcium current inside SR; NSR and JSR, network SR and terminal cisterns of SR. TC, TMC, CM, and CQ designate troponin, troponin-Mg sites, calmodulin, and calsequestrin.

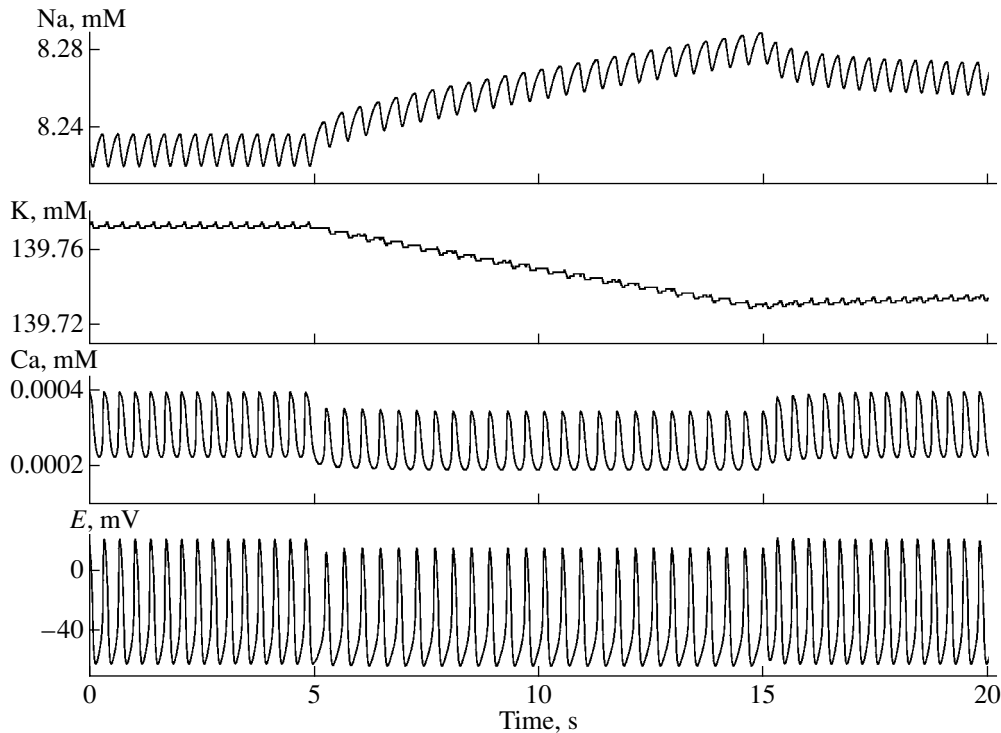


Fig. 2. Effect of acetylcholine on intracellular concentrations of Na^+ , K^+ , and Ca^{2+} and on the transmembrane potential E . The concentration of acetylcholine was 25 nM within the time interval of 5 to 15 s and 0 in any other time.

characteristic time of changes in the concentration of the major ions in the cell (Figs. 2–4). The great characteristic time of steady state onset for Na^+ and K^+ is determined by the fact that the final steady state of the major electrophysiological characteristics (such as amplitude, oscillation period, etc.) is reached during tens of seconds. Such times are usually observed in

vagus stimulation or acetylcholine perfusion/reperfusion. Gradual changes are usually attributed to slow diffusion of acetylcholine and its involvement a chain of biochemical reactions. Without doubts in the correctness of such explanations, it should be noted that the onset of intracellular homeostasis, described in this work, contributed to the function of the sinus node.

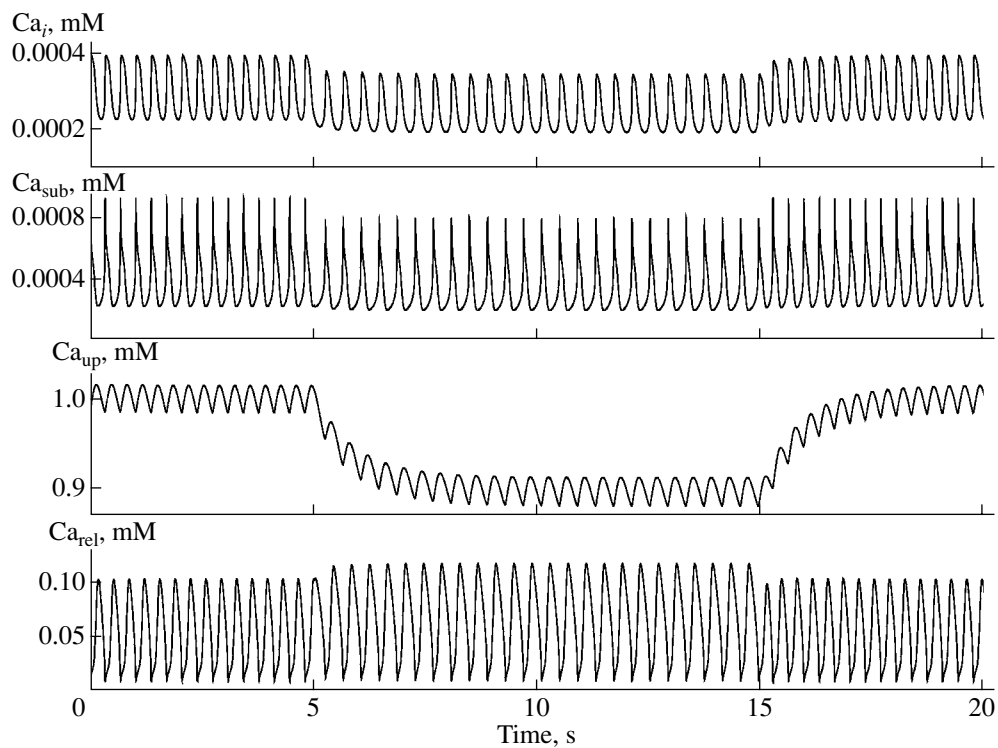


Fig. 3. Effect of acetylcholine on the concentration of Ca^{2+} in the cytoplasm (Ca_i), dyadic space (Ca_{sub}), in NSR and JSR (Ca_{up} and Ca_{rel}). The concentration of acetylcholine was 25 nM within the time interval of 5 to 15 s and 0 in any other time.

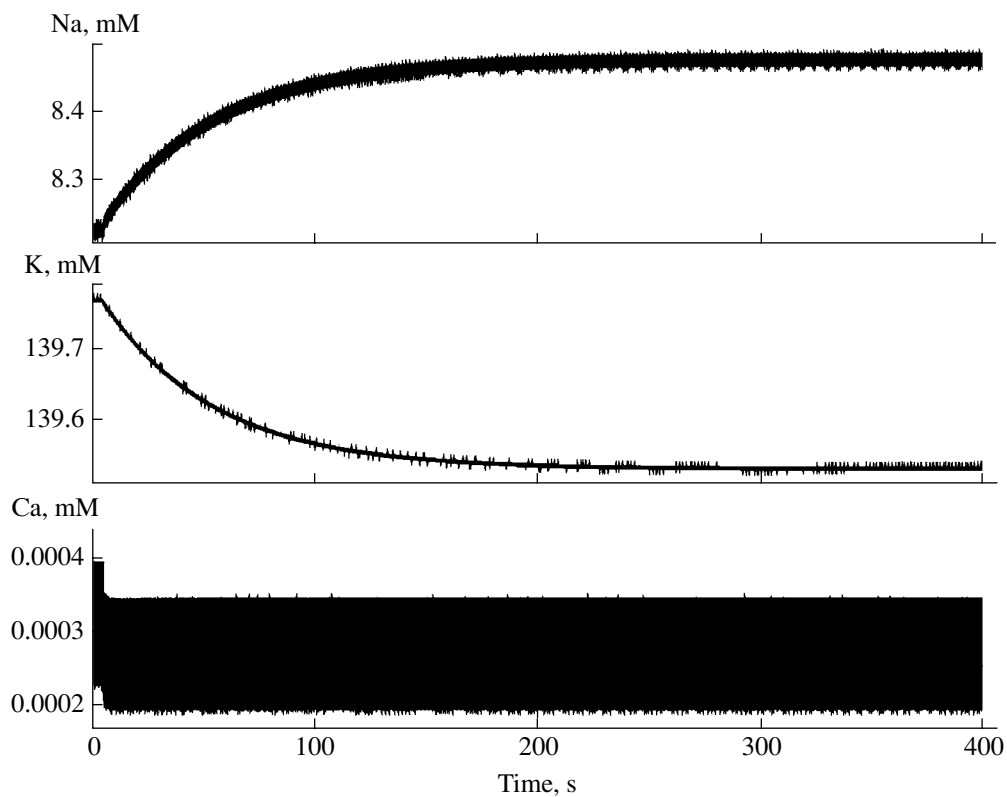


Fig. 4. Onset of intracellular homeostasis under exposure to acetylcholine. The concentration of acetylcholine was 25 nM within the time interval of 5 to 400 s and 0 in any other time.

The changes in the concentration of Na⁺ and K⁺ under exposure to 25 nM acetylcholine are relatively small (Fig. 4) in terms of percentage and effect on the steady-state Nernst potentials for these ions. However, such slight changes may result in considerable qualitative effects (e.g., in the occurrence of preautomatic pause [6]).

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