

Study of the Effect of Acetylcholine on the Excitability 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 the previous study, we investigated the effect of small doses of acetylcholine on spontaneous activity in sinus-node cells [1]. At high concentrations of acetylcholine, spontaneous activity was ceased. Here, we studied the effect of acetylcholine on the excitability of cells of true pacemakers of the sinus node of the rabbit after lacking spontaneous activity by them. It is shown that true pacemakers retain electrical excitability at acetylcholine concentrations up to 10 μ M. The excitation threshold with respect to current in dependence on the conductance of L-type calcium channels, concentration of acetylcholine and extracellular potassium ions has been determined, and the transmembrane rest potential has been measured. A nonlinear dependence of the excitation threshold on acetylcholine concentration was demonstrated, and an explanation to this phenomenon in terms of ionic currents is suggested.

Conditions of numerical experiments. The descriptions of the model of the sinus-node cells, effect of acetylcholine, and methods of numerical integration used in this study were described in [1–3]. In this work, when simulating acetylcholine-induced termination of spontaneous activity, measurements were taken after reaching the steady state (5–10 s). The cell was regarded excitable if stimulation caused membrane

depolarization to -10 mV or higher. The excitation threshold was estimated with an accuracy of 1 pA.

Results. An example of electric stimulation of sinus-node cells after the loss of spontaneous activity in the presence of 1 μ M acetylcholine is shown in Fig. 1. It can be seen that a weak current (0.01 nA) did not cause marked changes in membrane potential. A stronger yet underthreshold current (0.055 nA) caused a slight depolarization (up to -40 mV). A slight increase in the stimulatory current (to 0.07 nA) resulted in a full-scale response and complete membrane depolarization (up to $+20$ mV). Excitation threshold, whose crossing led to the full-scale response, in this case constituted 0.058 nA. Note that such time course is characteristic of excitable cells of functioning myocardium.

The excitation threshold increased as the conductance of L-type calcium channels increased (Fig. 2) and the concentration of extracellular potassium decreased (Fig. 3). The dependence of the threshold on the concentration of acetylcholine was not monotonous, which

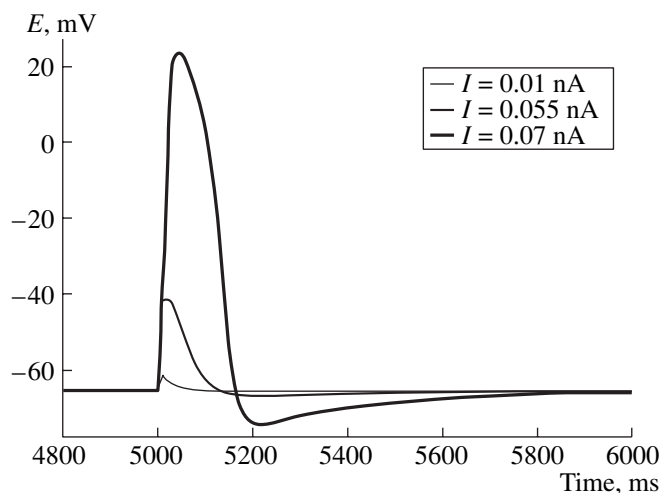


Fig. 1. Response of a true pacemaker cell after cessation of spontaneous activity induced by 1 μ M acetylcholine. Curves illustrate under-threshold (0.01 nA), near-threshold (0.055 nA), and above-threshold (0.07 nA) stimulation.

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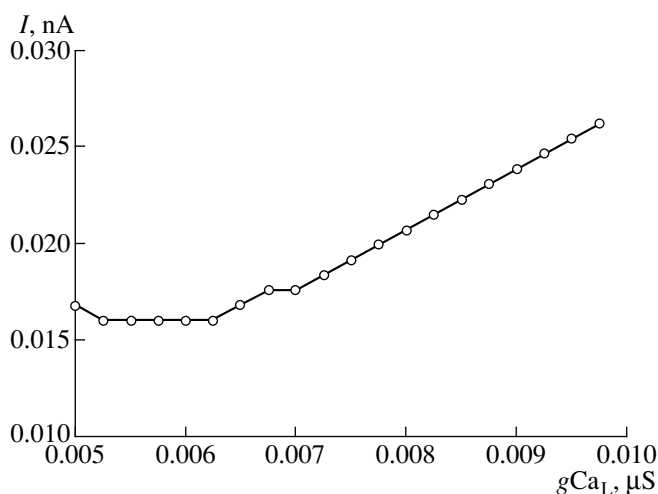


Fig. 2. Dependence of the threshold stimulatory current on the conductance of L-type calcium channels.

manifested especially pronouncedly in hyperkalemia (Fig. 3). For example, at an extracellular potassium concentration of 10.8 mM, the excitation threshold decreased at acetylcholine concentrations lower than 0.2 μ M and increased at greater acetylcholine concentrations. To account for the nonmonotonous pattern of threshold behavior, we determined the dependence of steady-state transmembrane potential under the same conditions (Fig. 4). It appeared that the steady-stage potential decreased with a decrease in extracellular potassium and increase in acetylcholine concentration. Hyperkalemia and high concentrations of acetylcholine caused membrane hyperpolarization; for example, at

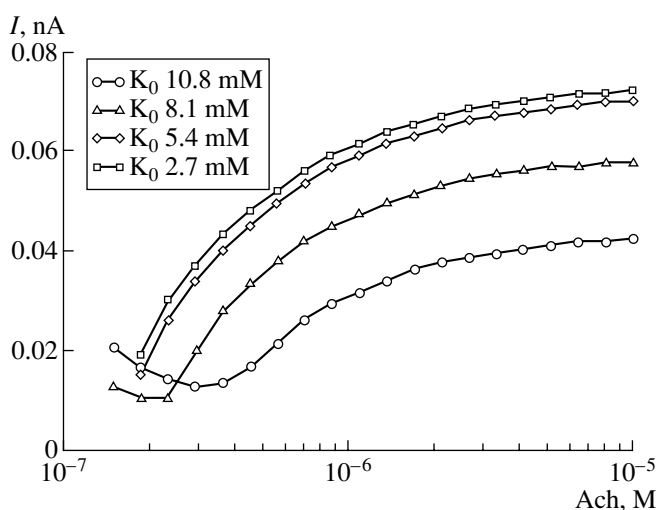


Fig. 3. Dependence of the threshold stimulatory current on acetylcholine concentration at various extracellular potassium concentrations (K_0). Hereinafter, Ach designates acetylcholine.

$K_0 = 2.7$ mM and 10 μ M acetylcholine, transmembrane potential reached -72 mV. In hyperkalemia and low acetylcholine concentrations, hyperpolarization of membrane was observed, and the transmembrane potential reached -37 mV (Fig. 4). At such potential, L-type calcium channels are partly inactivated [2].

Apparently, in the case of partial inactivation of calcium channels, greater stimulatory current for excitation of cells is required. This accounts for an increased excitation threshold at acetylcholine concentrations lower than 0.2 μ M (Fig. 3). At higher concentrations, the hyperpolarizing effect of acetylcholine is observed, the steady-state transmembrane potential becomes more negative; this withdraws calcium channels from the state of partial inactivation and enables excitation of cells by decreasing the excitation threshold. Further increase in acetylcholine concentration increases the excitation threshold due to $I_{Ca,L}$ suppression as a result of effect of acetylcholine on this current [1, 3] and membrane hyperpolarization.

Discussion. The computer-based model of single cells of true pacemakers of rabbit sinus node was used to study the excitability of cells after the loss of spontaneous activity induced by acetylcholine. A nonmonotonous dependence of the excitation threshold on acetylcholine concentration has been demonstrated. This dependence is observed under normal conditions; however, it is most pronounced in hyperkalemia. This phenomenon may play a key role in neurogenic fibrillation of ventricles [4], because the secretion of acetylcholine by postganglionic parasympathetic termini produces a

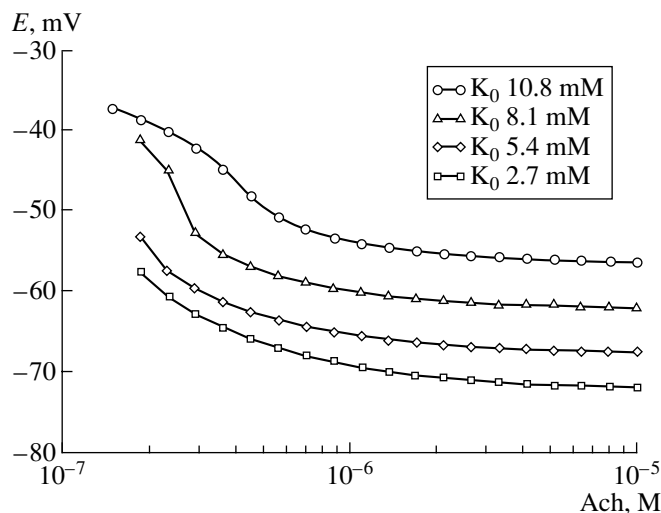


Fig. 4. Dependence of the steady-state transmembrane potential on acetylcholine concentration at various extracellular potassium concentrations (K_0).

gradient in acetylcholine concentration, which results in different nonmonotonous dependence of the excitation threshold on the distance to termini (i.e., to inhomogeneity of the myocardium). The arrhythmogenic role of inhomogeneous myocardium is well known.

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