

Study of the Effect of Acetylcholine on Ion Currents in Single Cells of True and Latent Pacemakers of Rabbit Sinus Node using Computer Simulation

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Acetylcholine (ACH), a neurotransmitter secreted by postganglionic parasympathetic termini, plays a key role in the regulation of spontaneous activity and excitation propagation in sinus node (SN) of mammals in normalcy and pathology. In this work, we used computer simulation to study the effect of ACH on currents of single cells of true and latent pacemakers of rabbit sinus node. It is shown that ACH differentially affects the spontaneous activity of pacemaker cells, depending on the maximal rate of action potential rise (dV/dt_{\max}). At $dV/dt_{\max} < 1.15$ V/s, spontaneous activity was stopped without deceleration of initial rhythm; at greater dV/dt_{\max} values, spontaneous activity was stopped against the background of deceleration of initial rhythm, which is related to different ratio of calcium currents $I_{Ca,L}$ and $I_{Ca,T}$.

Introduction. It is known that ACH secretion by postganglionic parasympathetic termini at vagus stimulation, as well as ACH perfusion and superfusion cause negative chronotropic effect, which may result in a complete suppression of spontaneous activity in SN [1–3]. Recent experiments showed that the termination of pacemaker activity in SN under exposure to ACH depends on the rate of action potential (AP) rise [3]. In SN pacemaker cells, the main effects of ACH are suppressing the calcium current $I_{Ca,L}$, activating the muscarinic potassium current I_{Kach} , and shifting the activation curves of the current I_f [4]. Changes in the function of these ACH-sensitive channels result in changes in action potential development, which, in turn, causes changes in function of other channels, because the conductivity of most of them depends on potential and time. Thus, to study the effect of ACH on pacemaker activity, simultaneous recording a large number of ion current is required, which cannot be done experimen-

tally. In this work, the effect of ACH on SN cells was studied using computer simulation.

Conditions of numerical experiments. To simulate true and latent pacemakers, we used the model based on rabbit SN cells, which has been described in detail in [5]. Simulating the effect of ACH on the currents $I_{Ca,L}$, I_{Kach} , and I_f was performed in accordance with the algorithms presented in [4]. Slight improvements of the model, which appeared after publishing [4, 5], were taken into consideration owing to a personal consultation of Prof. H. Zhang. An equivalent electric scheme of the model is shown in Fig. 1. To simulate transmembrane potential and 12 main currents, an original algorithm has been developed, and a special software on the language C has been written. Differential equations of simple relaxation $dx/dt = (x_{\infty} - x)/\tau$, which are included in descriptions of the majority of currents that function according to the Hodgkin–Häxly mechanism, were solved by direct integration, assuming that the coefficients $x(t + \Delta t) = x_{\infty} - (x_{\infty} - x)(t)\exp(-\Delta t/\tau)$ were constant. The other equations were integrated by the method of Euler. In general, integration scheme corresponded to the first order of accuracy. In calculations, we used automatically slaving pitch that varied in time within a range of 1–300 μ s; integration was performed over 4–8 s.

To study the effect of ACH in dependence on the rate of action potential rise, we modeled cells of true pacemakers, in which the $I_{Ca,L}$ current accounted for 52 to 98% of the overall calcium current ($I_{Ca,L} + I_{Ca,T}$). In normal state, this value is approximately 80% [2]. In the model, we varied the maximal conductivity of the corresponding currents rather than the currents themselves, which asynchronously change in time. Typical values of these parameters in cells of true pacemakers were 7.6 and 2.1 nS for $I_{Ca,L}$ and $I_{Ca,T}$, respectively.

Results. When simulating the effect of ACH on single cells of true and latent pacemakers, we observed qualitatively similar effects. In both cases, a negative chronotropic effect, hyperpolarization of membrane, as well as a decrease in amplitude and rate of rise were recorded. Figure 2 shows transmembrane potential and currents in a cell of true pacemaker under the exposure

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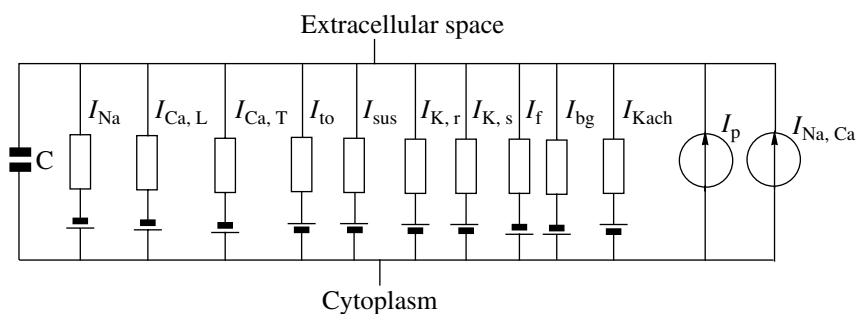


Fig. 1. Equivalent electrical scheme of the model of rabbit sinus node cells.

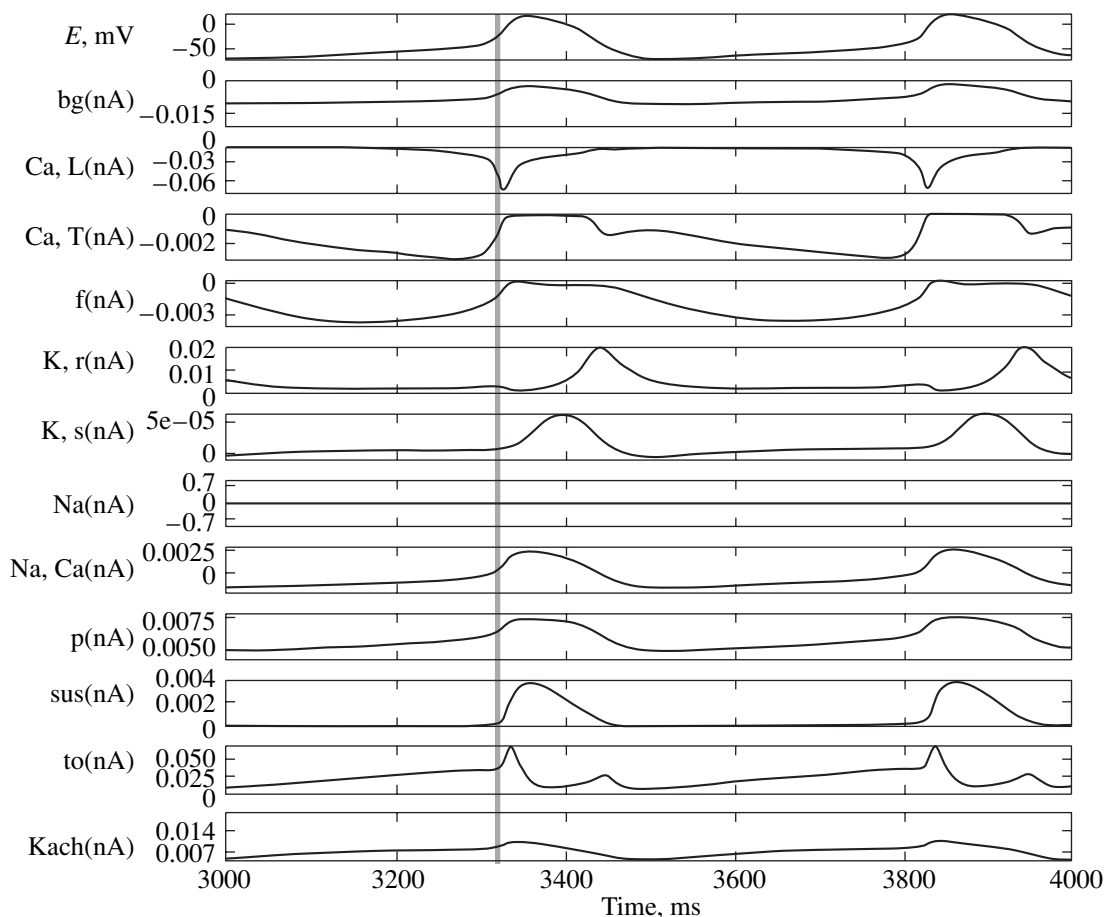


Fig. 2. Transmembrane potential (E) and currents (I_{bg} , $I_{Ca,L}$, $I_{Ca,T}$, I_{bg} , $I_{K,r}$, $I_{K,s}$, I_{Na} , $I_{Na,Ca}$, I_p , I_{sus} , I_{to} , and I_{Kach}) in the cell of true pacemaker of rabbit sinus node under exposure to $0.1 \mu\text{M}$ ACH. Vertical line at the moment $t = 3320$ ms designates the moment of maximal rate of AP rise.

to $0.1 \mu\text{M}$ ACH, which induced an increase in the period of spontaneous excitations from 0.31 to 0.5 s.

In true pacemakers, rapid sodium current is either absent or weakly pronounced. Unlike latent pacemaker and working cardiomyocytes, in cells of true pacemakers the rise-up AP portion is formed by a slower L-type calcium current rather than by a rapid sodium current (Fig. 3). This results, in particular, in a much lower rate

of AP rise in true pacemakers. A greater $I_{Ca,L}$ current increased the maximal depolarization rate in the rise-up AP portion, which was measured as dV/dt_{max} . The abscissa in Fig. 4 shows the corresponding values of the rate of AP rise. It is seen that the maximal ACH concentration, at which spontaneous activity was still observed, was higher in the case of cells with a greater rate of AP rise (Fig. 4, upper curve). An exception to

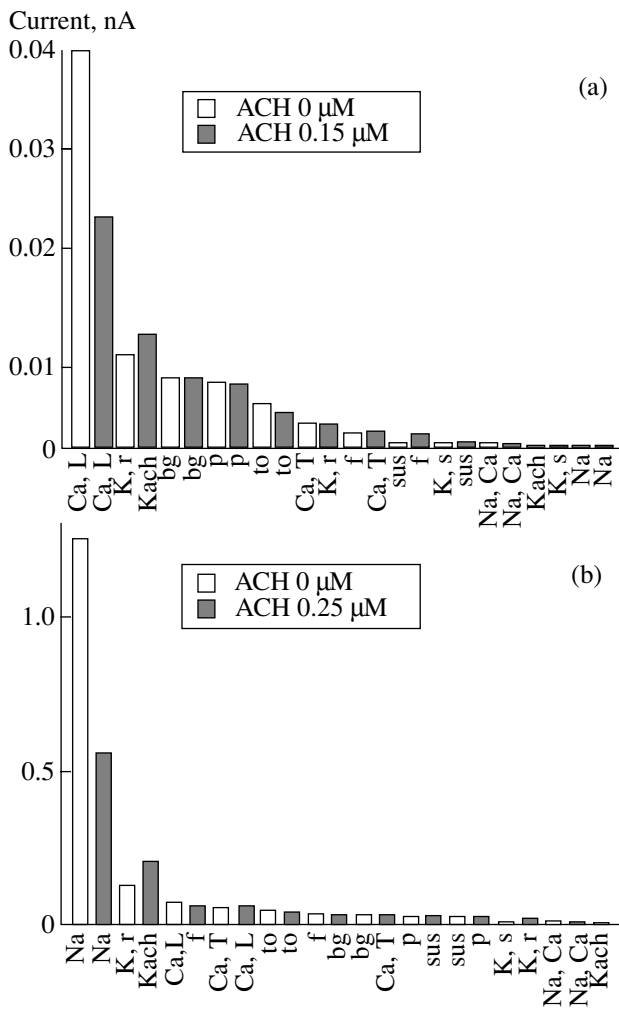


Fig. 3. Rank-order (with respect to strength) absolute values of currents at maximal rate of AP rise in the cell of (a) true and (b) latent pacemaker. White columns show the control; hatched columns show the effect of ACH. The greatest current was observed in the case of $I_{Ca,L}$ (a) and I_{Na} (b). In the last case, $I_{Ca,L}$ is only slightly involved in AP rise formation.

this tendency was two rightmost points, in which a slight decrease in the maximal ACH concentration was observed. This phenomenon may be due to an abnormally great disproportion in L- and T-type calcium currents (in the last point, $I_{Ca,L}$ current accounted for 98% of overall calcium current).

Figure 4 also shows the dependence of the period of spontaneous activity of pacemaker cells on the rate in their AP rise under exposure to maximal ACH concentration (medium curve) and in the control (lower curve). It is seen that, in the control, this period practically did not depend on the rate of AP rise. This may be explained by the fact that the $I_{Ca,L}$ current, which forms the AP rise (Fig. 3a), was rapidly inactivated and was involved in AP repolarization and slow diastolic depolarization only to a little extent.

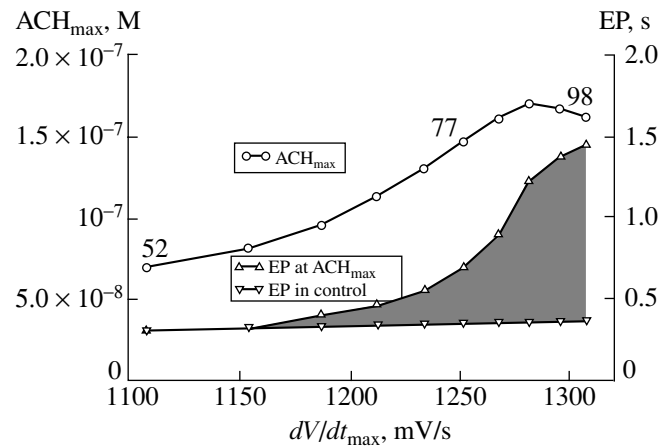


Fig. 4. Dependence of ACH concentration that leads to a loss of spontaneous activity (upper curve) and excitation period (EP) at maximal ACH concentration (medium curve) and in the absence of ACH (lower curve) on the rate of AP rise in the cell of a true pacemaker. Excitation periods almost coincide at a small rate of AP rise and significantly disperse as the rate of AP rise increases. Hatched area shows the range of changes in excitation period. Numerals on the upper curve show the minimal, normal, and maximal proportion of $I_{Ca,L}$ in calcium current (%).

In the presence of ACH, the period of activity at a rate of AP rise of 1.3 V/s increased almost by 400%. At a smaller rate of AP rise, the chronotropic effect was less pronounced and decreased to zero at dV/dt less than 1.15 V/s (Fig. 4). These data allowed us to conclude that there are two different scenarios of ACH action on true pacemaker cells: (1) termination of spontaneous activity after a period of rhythm deceleration and (2) termination of spontaneous activity without preceding rhythm deceleration. The first and second scenarios are characteristic of cells with a great and small rate of AP rise, respectively. When simulating the activity of latent pacemakers under exposure to ACH, only the first scenario was observed.

To understand these phenomena, consider in more detail the effect of ACH on the currents $I_{Ca,L}$, I_{Kach} , and I_f and the role of these currents in AP formation. For true pacemakers, $I_{Ca,L}$ is the main current that forms the AP rise (Fig. 3a). Due to suppression of this current, depolarization rate is decelerated. When the threshold maximal ACH concentration is exceeded, a complete suppression of AP rise and own activity of true pacemakers is observed. Apparently, at increased $I_{Ca,L}$ current, higher ACH concentrations are required for its suppression. This assumption accounts for the dependencies shown in Fig. 4: an increase in $I_{Ca,L}$ current led to a greater rate of AP rise, which required a higher ACH concentration to suppress spontaneous activity (Fig. 4, upper curve). Currents I_{Kach} and I_f only slightly affected the formation of AP rise; however, under exposure to ACH, these currents cause a negative chronotropic effect. Thus, the first scenario of ACH action (namely,

suppression of spontaneous activity after a period of rhythm deceleration) takes place in this case. In the second scenario, ACH affects the $I_{Ca,L}$ and I_f currents less significantly than the I_{Kach} current [1]. H. Zhang *et al.* showed that deceleration of sinus rhythm under exposure to ACH is determined predominantly by the current I_{Kach} [4]. Therefore, in cells with small $I_{Ca,L}$ current, it will be significantly suppressed already at low ACH concentrations, at which the activation of I_{Kach} is still not significant. In this case, the termination of spontaneous activity under exposure to ACH will take place earlier than deceleration of rhythm occurs (Fig. 4, at $dV/dt_{max} < 1.15$ V/s).

Conclusion. The computer model of single cells of true and latent pacemakers of rabbit sinus node corroborated both the well-known data on the effect of ACH on pacemakers and recent data on the dependence of the ACH effect on the rate of AP rise [3]. This model made it possible to discover a new regime of termination of spontaneous activity without preliminary deceleration of rhythm, which has not been found experimentally. The main advantage of mathematical simulation is the possibility of simultaneous evaluation of all main cell currents, as well as the transmembrane poten-

tial and its derivative at an arbitrary time. Thus, modern methods of computer simulation become a potent reliable tool for studying complicated physiological systems.

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