The Negative Chronotropic Effect of Cs^+ Ions on Generation of Transmembrane Potentials in Mouse Sinoatrial Node Cells

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Experiments on spontaneously contracting strips from sinoatrial region of the hearts of 2-month-old albino mice showed that cesium (Cs^+), a blocker of hyperpolarization-activated I_f current, in a concentration of 1 mM produced the greatest negative chronotropic effect on the duration of diastolic depolarization phase (75%), its rate (59%), and action potential duration (29%). The threshold concentration of Cs^+ was approximately 0.15 mM. In a concentration of about 8.5 mM, spontaneous generation of action potentials stopped. The effect was reversible. Thus, blockade of I_f current by Cs^+ reduced the rate of action potential generation in cells of mouse sinoatrial node by ~42% in comparison with controls.

Key Words: *sinoatrial node; transmembrane potential; cesium; mouse*

The structure of mouse sinoatrial node (SAN) had been studied using immunohistochemical techniques and light microscopy [3,5]. At the same time, the data on the basic parameters of action potentials (AP) of pacemaker cells in mouse SAN are scanty and contradictory. Thus, the mean AP amplitude varies from 55 mV [5] to 79 mV [4], and the values of maximum rate of depolarization (dV/dt_{max}) from 6-27 V/sec [5] to 48 V/sec [4]. These discrepancies can be explained by the fact that mouse SAN consists only of several hundred (400-600) pacemaker cells 3-6 microns in diameter and 25-30 μ in length. It should be mentioned that SAN cells spontaneously beat with high frequency and are surrounded by the connective tissue, which complicates AP registration with glass microelectrodes. In experiments on isolated SAN cells, the researcher cannot determine the original localization of the cell in SAN area [2,4]. Therefore, the first aim of our study was to determine the most probable localization of SAN cells region using microelectrode mapping on the endocardial surface.

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Cesium (Cs^+) is the most common blocking agent of hyperpolarization-activated I_f current. In isolated SAN cells, Cs^+ (1-2 mM) reduced the frequency of AP generation in rabbits by 12-30% [2] and in humans by 26% [6] in comparison with the control. Similarity of genomes and principle of organization and functioning of mouse and human hearts explains increasing interest to studying of the mechanisms of pacemaker activity generation in mouse SAN. However, we found no published data on the effect of Cs^+ on the basic AP parameters in mouse SAN cells. To fill this gap, we studied the effects of Cs^+ in concentration range from 0.1 to 10 mM on the configuration of transmembrane AP in cells with dV/dt_{max} from 4 to 13 V/sec in mouse SAN. Based on the data [2-4,6], we assumed that Cs^+-sensitive I_f current could be an active contributor to AP generation in mouse SAN cells.

MATERIALS AND METHODS

Experiments were carried out on spontaneously contracting strips from the sinoatrial region of the hearts of 2-month-old male albino mice (n=21) weighing 30±5 g. The rats were anesthetized with ether and sac-

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rificed by cervical dislocation. The chest was opened, and the heart was quickly removed. The preparation was 3×2 mm in size and included the region between *v. cava inferior* and *v. cava superior* and a portion of *crista terminalis* (Fig. 1). Rhythmically beating stripes of the sinoatrial region were placed in a 5-ml chamber perfused with oxygenated saline containing (in mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgSO_4$, 10 glucose, and 5 HEPES; pH 7.4 at 31±0.5°C.

We applied standard microelectrode techniques to study action potentials. Micropipettes with fiber (Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino) filled with 2.5 M KCl (initial resistance 20-30 MΩ) were used. The operating range of the amplifier was from 0 to 5 kHz. AP were recorded using an analog-to-digital converter (E14-140, L-CARD) to the computer hard drive.

The following AP parameters were measured and analyzed: action potential amplitude, maximum diastolic potential, spontaneous depolarization, duration of action potential at 50% (APD_{50}), 90% (APD_{90}), and100% (APD_{100}) repolarization, cycle length, maximum rate of AP rise during phase 0 (dV/dt_{max}), and the rate of diastolic depolarization. The boundary between the diastolic depolarization (phase 4) and fast depolarization (phase 0) was determined graphically: tangents were built and the perpendicular was constructed from their intersection.

For statistical analysis, the mean value of the parameters in each experiment was used (n=5-8 cells), the arithmetic mean±standard deviation (M±σ) was calculated using Microsoft Office Excel. The differences were checked by paired comparisons using Student's t-test. The differences with $0.01 \le p \le 0.05$ were considered significant.

RESULTS

Electrophysiological mapping was started from the center between the lateral and medial branches of *crista terminalis* at an interval of about 50 μ by moving the electrode along lines parallel to the *crista terminalis*. Recorded AP were arbitrarily divided into three configuration types (Fig. 1; Table 1). Type I, AP with the slowest rapid depolarization rate (dV/dt_{max}<13 V/sec; 11 strips), low amplitude of AP and overshoot, maximum APD_{90}, and rapid diastolic depolarization (Fig. 1, *a*; Table 1). We believed that these AP are generated by cells working in the regimen of true pacemakers. Such AP configurations were registered in spontaneously beating strips with the endocardial side exposed up in the center between *v. cava inferior* and *v. cava superior* at a distance of about 0.2-0.3 mm from the *crista terminalis* (Fig. 1).

If the microelectrode was shifted to 50-100 μ towards *v. cava inferior*, pacemaker AP were recorded with faster dV/dt_{max} (~46 V/sec; 9 strips). They belonged to type II with greater amplitude of AP and overshoot, shorter AP, and lower rate of diastolic depolarization than in type I cells (presumably the cells of the central part of the SAN; Fig. 1, *b*; Table 1). Outside the area marked in Figure 1 with circles

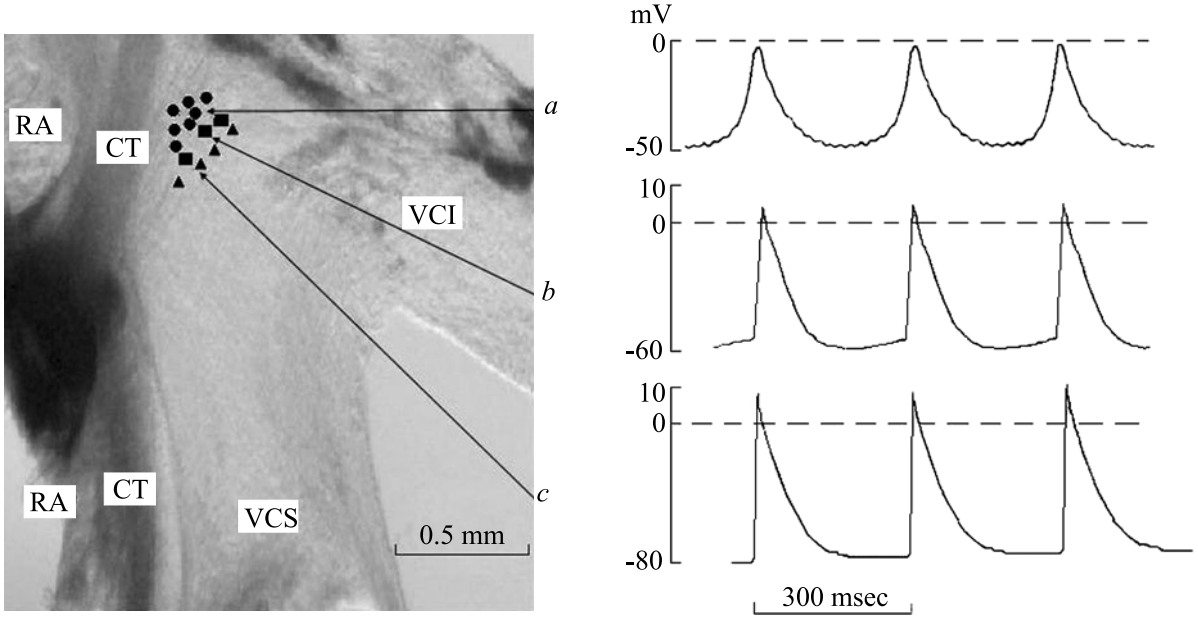

Fig. 1. The preparation of mouse sinoatrial region (view from subendocardium) and type III AP. *a*) AP in true pacemaker cells; *b*) latent pacemaker cells; *c*) myocardial contractile cells of the right atrium. VCS: *vena cava superior*; VCI: *vena cava inferior*; CT: *crista terminalis*; RA: right atrium. Circles: sites of AP recording in true pacemaker cells; squares: sites of AP recording in latent pacemaker cells; triangles: sites of AP recording in atrial myocardial contractile cells.

TABLE 1. Electrophysiological Properties of AP Cells in Mouse Cardiac Sinoatrial Region Recorded on the Endocardial Surface ($M\pm\sigma$)

Parameter	True pacemaker cells (n=11)	Hidden pacemaker cells (n=9)
AP, mV	39±5*	62±11
Spontaneous depolarization, mV	10±3*	7±1
APD_{50}, msec	78±10	72±9
APD_{90}, msec	137±13	129±11
APD, msec	279±17	283±15
Diastolic depolarization, msec	105±21*	97±26
$dV/dt_{макс}$, V/sec	8±3**	46±20
V_4, mV/sec	99±21	73±18

Note. APD_{50} and APD_{90}: duration of action potential at 50% and 90% repolarization; APD: total duration of action potential; V_4: rate of slow diastolic depolarization (phase 4); n: number of stripes. *p<0.05, **p<0.01 compared with latent pacemaker cells.

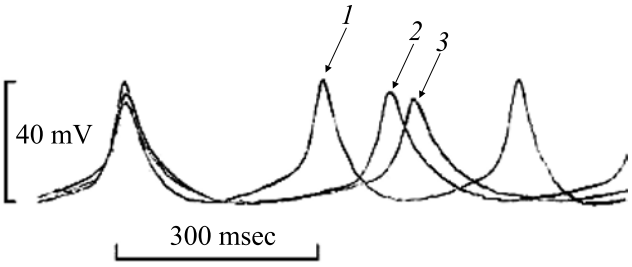

Fig. 2. Changes in AP configuration in a cell of mouse SAN with $dV/dt_{max}\approx$6 V/sec after addition of 1 and 3 mM Cs^+ to the perfusate. Deceleration of diastolic depolarization, minor reduced AP amplitude, and increasing the length of final repolarization phase were registered. The effect was reversible. *1*) control; *2*) 1 mM Cs^+; *3*) 3 mM Cs^+.

and squares, only type III AP were recorded, *i.e.* cells without spontaneous depolarization phase like atrial contractile myocardium. It should be noted that AP of atrial type were repeatedly recorded on the endocardial surface within the area marked as putative SAN region. Our results are consistent with the previous data [1] obtained on rat sinoatrial region recorded on the epicardial surface.

Thus, new data were obtained on the localization of cells working in the regimen of true and latent pacemakers in mouse SAN. Their basic electrophysiological characteristics were clarified; for example, the amplitude of AP in cells working in the regimen of true and latent pacemakers, *etc*. (for comparison – [3] and [5]). We suppose that mouse SAN is characterized by unifocal type of pulse generation.

It should be noted that the duration and mean rate of spontaneous depolarization (phase 4) were the most Cs^+-sensitive parameters. The graph representing the changes in electrophysiology depending on the concentration of Cs^+ showed that the threshold concentration of Cs^+ is about 0.15 mM (n=4). Cs^+ (1 mM) most strongly reduced the duration and rate of diastolic depolarization phase (Figs. 2 and 3): 73 and 58%, respectively. Interestingly, the duration of AP at 90% repolarization (APD_{90}) also increased by on average 20%, and finally the rate of AP generation in cells working in regimen of true pacemakers slowed by an average of 42±12% (n=6) in comparison with the control. Increasing Cs^+ concentrations in bathing solution from 1 to 3 and 5 mM did not reduce significantly the rate of diastolic depolarization (p>0.05; n=5). In this case, the rate of AP generation decreased by 10-15% compared to that at 1 mM Cs^+ due to lengthening of diastolic depolarization and APD_{90} by 5-10%. Based on these results we assume that Cs^+ (1 mM) blocks I_f current by 85-90% in comparison with the control.

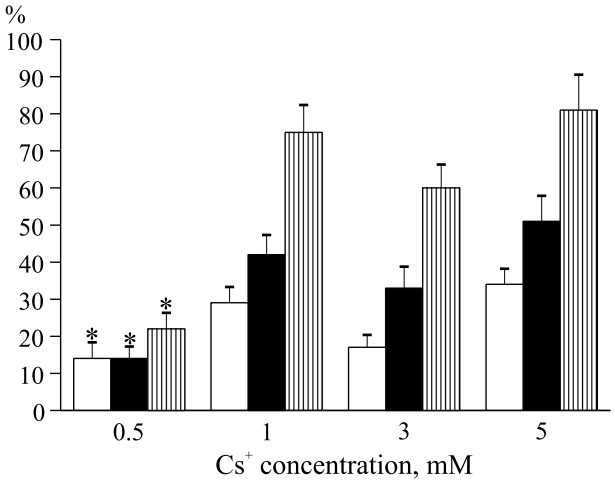

Fig. 3. Effects Cs^+ on AP parameters. Light bars, APD90; dark bars, total duration of the action potential; hatched bars, diastolic depolarization depending on the Cs^+ concentration (percentages relative to control cells). *p<0.05 compared with 1 mM Cs^+.

Increasing Cs^+ concentrations from 5 to 7.5 and 10 mM terminated spontaneous AP generation in mouse SAN cells (on average, in a concentration of 8.6±1.0 mM; *n*=4) due to blockade of rapid depolarization phase after 5-min exposure. Electrical activity of pacemaker cells was partially restored at the 1st minute of washout. The effect of high Cs^+ concentration was reversible after 40-50-min perfusion in normal saline.

Thus, in mouse SAN cells with mean dV/dt_{max} = 8 V/sec, Cs^+ (1 mM), a blocker of hyperpolarization-activated I_f, decreased diastolic depolarization rate by 58% and frequency of AP generation by 42% in comparison with the control. Cs^+-sensitive current makes a significant contribution (but is not the only contributor) to generation of diastolic depolarization [2,4]. At higher concentrations (>3-5 mM) Cs^+ ions lose their specificity and can block other cation channels.

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