Modelling of the Effects of Na^+ and Ca^{2+} Ions on the Generation of Action Potentials by Rabbit Heart True Pacemaker Cells

V. A. Golovko[1] and I. Tipāns[2]

1 *Institute of Biology, Komi Branch of the Academy of Sciences of the USSR, 167610 Syktyvkar, USSR*

2 *Riga Polytechnical Institute, Lenin str. 1a, 226355 Riga, USSR*

Abstract. A comparison of results of modelling and physiological experiments allowed to estimate in the first approximation the quantitative contribution of calcium and sodium ions to the generation of different ionic currents determining the shape of the action potential (AP). Generally, the model reproduced adequately variations in AP shape with changes in the external concentrations of Na^+ and Ca^{2+}. At the same time the model differs slightly from the experimental results. To improve the model kinetic variables of slow inward current have to be taken into account.

Key words: Pacemaker cells — Modelling — Na^+, Ca^{2+} effects

Introduction

There are several facts suggesting that Na^+ and Ca^{2+} ions have non-standard effects on heart sinoatrial region cells. According to Toda (1969) a decrease in the external concentration of Ca^{2+} resulted in a shortening of AP peak in a rabbit heart. Noma and Irisawa (1976 a) and Op't Hof et al. (1980) reported a similar condition to prolong the duration of AP peak or to have no effect on it. The reasons for non-standard variations of the duration of AP peak and frequencies of spontaneous heart beats in response to changes in Na_o^+ and Ca_o^{2+} are not yet clear. It has been suggested that during the generation of AP, Na^+ and Ca^{2+} ions regulate several processes. For example, with Na^+ ions replaced by K^+, Ca^{2+} regulates K^+ outflow (Lipsius and Vassalle 1978; Brown and Di Francesco 1980).

We employed the model of AP generation by the true pacemaker cells in order to quantitatively estimate the interaction of ionic currents and to predict the nature of variations in the main AP parameters as a result of the action of Na^+ and Ca^{2+}

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ions. Data obtained in computer experiments were compared with results of physiological experiments carried out on strips of the sinoatrial region of rabbit hearts.

Theory of the model

Fig. 1 shows a schematic representation of the so far known major ionic events responsible for AP generation. The fast depolarization phase of true pacemaker cells is determined by the slow inward current I_{si} carried mainly by Ca^{2+} or Na^+ ions. They are transfered due to a concentration gradient during the opening of selective ionic channels. The repolarization phase begins when the membrane potential reaches a level of +10 mV. Slow inward current is inactivated, and 20—30 ms later, the outward going and time dependent current I_K grows and flows in direction of the concentration gradient (Noma and Irisawa 1976b; Brown et al. 1979). After AP peak has been generated, ionic concentrations of Na^+, K^+ and Ca^{2+} reach their initial levels as a result of active ionic mechanisms operating during the diastole.

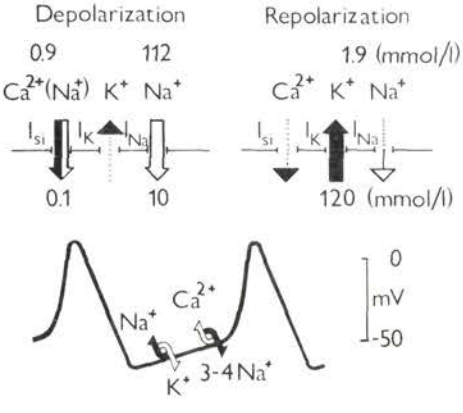

Fig. 1. Schematic representation of the model of membrane ionic currents reproducing full time course of ionic movements in a pacemaker cell. Interlaced arrows denote ionic pumps; arrows indicate passive ionic transfer; numbers indicate extracellular (*top line*) and intracellular (*bottom line*) concentrations of ions at the beginning of the depolarization in a rabbit heart cell. I_{si} — slow inward current; I_{Na} — fast inward current; I_K — outward current.

Materials and Methods

The reconstruction of pacemaker AP was done by integrating a set of seven differential equations (Yanagihara et al. 1980; Bristow and Clark 1982). The membrane model included a capacitance C_m shunted by several independent ionic channels for:

1) slow inward current; I_{si}
2) fast inward current; I_{Na}
3) outward current; I_K
4) hyperpolarization activated current; I_h
5) leakage current; I_l.

I_{si} and I_{Na} are described by two kinetic variables of the opening and closing of ionic channels. The equations were integrated untill periodic changes of the transmembrane potential were obtained. The

maximal (in modulus) value of potential E_{max} and kinetic variables corresponding to E_{max} served as initial conditions for the calculations. Using kinetic variables of channel conductance (membrane potential and time functions) we could reproduce with a sufficient correctness the shape of AP. The modelling of AP was carried out using an EC-1033 computer and a program NOIRI developed in the Riga Polytechnical Institute. In contrast to the procedure used by Yanagihara et al. (1980) we used the Runge-Kutta integration procedure with a variable step (Tipāns and Lavendels 1983). The entire period of AP generation involved 3—5 thousands integration steps and took 5—15 minutes to compute. Models of 11 different combinations of currents I_{si} and I_{Na} were considered.

Physiological experiments were carried out on spontaneously beating strips of the rabbit heart sinoatrial region (14 animals). Preparations were perfused with the Hank solution of the following composition (in mmol/l): NaCl 137; KCl 5.4; $CaCl_2$ 1.8; $NaHCO_3$ 16.7; NaH_2PO_4 1.0; KH_2PO_4 1.0; $MgSO_4$ 0.8; glucose 11. The solution was continously aerated. The decrease in Na^+ was compensated for by an equiosmotic amount of sucrose. The external concentration of Ca^{2+} was varied as follows: 1.8; 0.9; 0.45; 1.8; 3.6, pH = 7.4—7.6.

Preparations were let in the solutions of a given concentration for 30—40 minutes. Transmembrane AP were recorded using glass microelectrodes. Action potentials with maximal rates of rise ($v_{o,max}$) of less than 3 V/s were considered as belonging to true pacemaker cells (Kohlhardt et al. 1976; Bleeker et al. 1980; Golovko 1982).

Results

General characteristics of AP of true pacemaker cells

Transmembrane AP of different shapes were obtained from spontaneously beating strips of the rabbit heart sinoatrial node. Parameters of true pacemaker cells measured experimentally and those computed according to the model are shown in Table 1.

Effects of Ca_o^{2+}

In computer experiments succesive depression of I_{si} by 10; 20; 30; 40 or 45% at constant values of other currents resulted in a decrease in both the overshoot, $v_{o,max}$ and the repolatization rate v_3 (Fig. 2*A*). The rhythm of spontaneous beats became slowed and AP spikes prolonged. The most significant effect was observed when I_{si} decreased to 55 % of its normal value. A decrease of I_{si} by more than 45 % blocked the generation of AP. When I_{si} exceeded the normal value by 50 %, the overshoot increased from 20 to 25 mV. The maximal depolarization rate $v_{o,max}$ then increased from 3.6 to 6.1 V/s. In experiments with preparations exposed to low-Ca solution an overshoot decrease from +2.5 to −5 mV was observed when Ca_0^{2+} decreased from 1.8 to 0.45 mmol/l (Fig. 2*B*). Maximal depolarization rate decreased from 2.8 ± 0.2 to 1.3 ± 0.1 V/s. The effect of Ca^{2+} on AP generation was enhanced during the following 10—30 minutes.

An increase in Ca^{2+} concentration in the settling medium from 1.8 to 3.6 mmol/l resulted in an overshoot increase by about 10 mV; $v_{o,max}$ increased by

Table 1. Electrophysiological characteristics of true pacemaker cells of the sinoatrial region of rabbit heart.

Parameters Concentration* or current** variations	E_{max} (mV)	Overshoot (mV)	DAP_{100} (ms)	Duration of diastole (ms)	$v_{0,max}$ (V/s)
* 1.8 mmol/l Ca^{2+}; 137 mmol/l Na^+	-60 ± 2 $n=27$	2.5 ± 1.1 $n=27$	160 ± 10 $n=23$	200 ± 15 $n=23$	2.8 ± 0.2 $n=14$
* 1.8 mmol/l Ca^{2+}; 68 mmol/l Na^+	-58 ± 4 $n=11$	-11 ± 4 (b) $n=11$	180 ± 15 $n=7$	210 ± 10 $n=9$	1.7 ± 0.1 $n=10$
* 0.45 mmol/l Ca^{2+}; 137 mmol/l Na^+	-54 ± 3 $n=14$	-5 ± 3 $n=14$	150 ± 10 $n=7$	200 ± 20 $n=8$	1.3 ± 0.1 (c) $n=10$
* 3.6 mmol/l Ca^{2+}; 137 mmol/l Na^+	-51 ± 1 (b) $n=13$	13 ± 1 (b) $n=13$	170 ± 10 $n=8$	120 ± 20 (b) $n=7$	4.2 ± 0.2 $n=9$
* 3.6 mmol/l Ca^{2+}; 68 mmol/l Na^+	-42 ± 2 (a) $n=9$	5 ± 2 $n=9$	180 ± 25 (c) $n=9$	165 ± 40 $n=8$	1.9 ± 0.4 $n=7$
** $I'_{Na} = I_{Na}$; $I'_{si} = I_{si}$	-62	20	97	247	3.6
** $I'_{Na} = I_{Na}$; $I'_{si} = 0.8\, I_{si}$	-62	15	98	308	3.5
** $I'_{Na} = I_{Na}$; $I'_{si} = 0.55\, I_{si}$	-62	-7	127	605	0.9
** $I'_{Na} = 0.5\, I_{Na}$; $I'_{si} = I_{si}$	-62	21	97	307	4.0

* — physiological experiment; ** — modelling; E_{max} — maximal diastolic potential; DAP_{100} — duration of AP measured in 100 % repolarization time;
$v_{0,max}$ — maximal rate of depolarization; n — number of cells; I'_{Na} — varied fast inward current; I'_{si} — varied slow inward current.
(a) — $p<0.001$, (b) — $p<0.01$, (c) — $p<0.05$, compared with control.

about 50 % and the frequency of AP generation by about 10 %.

Effects of I_{Na}

In these series of model experiments the fast inward sodium current was Succesive depression of I_{Na} by 25; 50; 75 and 100 % of the normal value strongly affected the duration of diastolic depolarization (Fig. 3 *A*). The duration of the AP peak as a whole remained constant, however a slight increase in the fast depolarization phase and a shortening of the final repolarization by about 10 % was observed.

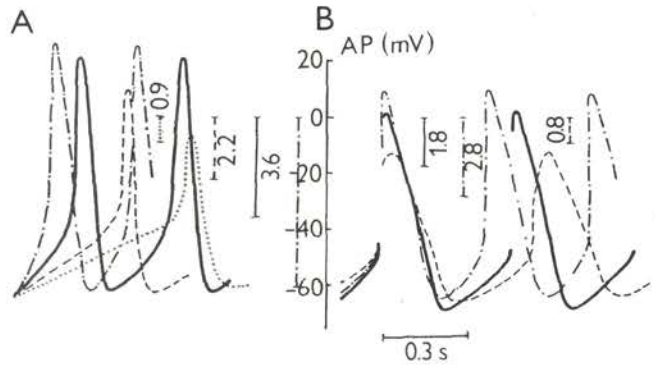

Fig. 2. Comparison of results obtained by modelling (*A*) during successive variation of I_{si} and those of physiological experiments (*B*) in a low-Ca medium. I'_{si} — varied current; *A*: full line — $I'_{si} = I_{si}$ (standard); dot-dashed line — $I'_{si} = 1.5\ I_{si}$; broken line — $I'_{si} = 0.7\ I_{si}$; dotted line — $I'_{si} = 0.55\ I_{si}$. *B*: full line — control (1.8 mmol/l Ca^{2+}); dot-dashed line — 3.6 mmol/l Ca^{2+}; broken-line — 0.45 mm/l Ca^{2+}.

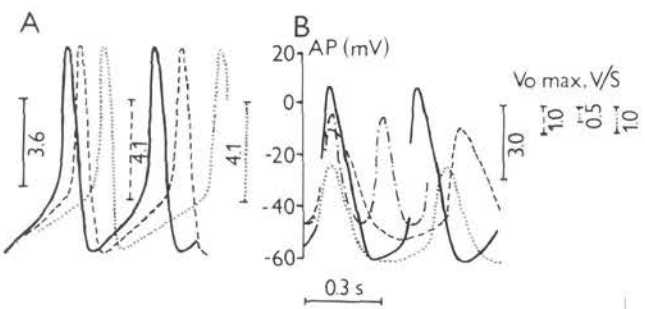

Fig. 3. Comparison of results obtained by modelling (*A*) during succesive decrease of I_{Na} and those of physiological experiments (*B*) in a low-Na medium I'_{Na} — varied current; *A*: full line — $I'_{Na} = I_{Na}$ (standard); broken line — $I'_{Na} = 0.75\ I_{Na}$; dotted line — $I'_{Na} = 0$. *B*: full line — control; broken line — 40 minutes exposure in Hank solution with 50 % NaCl; dot-dashed line — 10 minutes exposure in a solution with 200 % $CaCl_2$ + 50 % NaCl; dotted line — 5 minutes exposure in a solution with 50 % NaCl + 50 % $CaCl_2$.

Effects of sodium on the electrophysiological properties of true pacemaker cells were studied in a series of 9 experiments. In low Na solution (50 % NaCl) a decrease in E_{max}, overshoot and $v_{o,max}$ were observed 2—5 minutes after the exposure and AP generation rhythm slowed (Fig. 3 *B*). During the simultaneous removal of 50 % of $CaCl_2$ and NaCl from the perfusate a faster decrease of these parameters was recorded. When $CaCl_2$ concentration remained constant in low-Na solution (3.6 mmol/l), the values of overshoot and $v_{o,max}$ decreased less pronounced than in normal low-Na solution.

Discussion

Modelling of AP generation by the true pacemaker cells in rabbit heart showed that the Hodgkin-Huxley type mathematical models are useful for the analysis of ionic effects.

The slightly higher AP amplitude in the model as compared with the actually recorded AP is due to the parameters obtained in cells with $v_{o,max}$ from 3 to 10 V/s as studied by the authors (Yanagihara et al. 1980) of the mathematical model. We suppose that similar characteristics are mainly inherent to latent pacemaker cells. Generally, the model adequately reproduced variations in AP shape with varying external concentrations of Na^+ and Ca^{2+}.

The participation of Ca^{2+} and Na^+ ions in the generation of the fast depolarization phase has been supported by computer simulations. When I_{si} and I_{Na} were varied, $v_{o,max}$ increased or decreased according to the direction of the variation; this was in good agreement with physiological experiments. At the same time there were some discrepancies in the amplitude of variation. For example, an about twofold decrease in I_{si} resulted in the abolishment of AP generation in the model experiments. Under physiological conditions AP was still generated over 40—60 minutes when Ca^{2+} decreased about four times. The continuous generation of AP in low-Ca solution was probably due to calcium depot in the cells. On the other hand, a compensation for calcium by sodium ions is also possible. The present model considers the total slow inward current not considering the respective contributions of these ions. When the model fast inward current I_{Na} stepwisely decreased from 1 to 0.5 of its normal value, the diastole was prolonged by about 25 % and $v_{o,max}$ increased by about 10 %. In the actual experiment diminishing of Na_o^+ to one half of the normal value influenced all the parameters of AP: overshoot decreased by about 10 mV, $v_{o,max}$ decreased three times, the duration of the diastole increased by about 40 %. Thus, the effects of Na_o^+ decrease in the solution were actually more pronounced than in computer experiments. These discrepancies were in good agreement with facts concerning the role of Na^+ in the generation of the slow inward current (Toda 1969; Lipsius and Vassalle 1979; Brown et al. 1979).

One major question concerning the investigation of generating mechanisms of AP in pacemaker cells is the quantitative estimation of the respective contributions of Na^+ and Ca^{2+} to I_{si}. Figure 4 shows variations of $v_{o,max}$ resulting from decreasing the coefficient of I_{si} and varying the external concentrations of Na^+ and K^+.

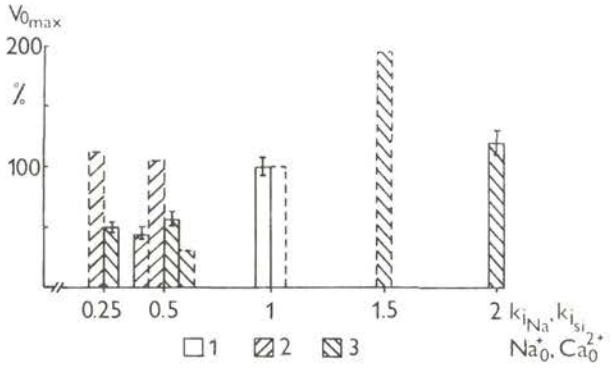

Fig. 4. Diagram of variations in $v_{o,max}$ with varied currents I_{si} and I_{Na} in the model and external concentrations of Na^+ and Ca^{2+} in physiological experiments. Full line — experiment; broken line — modelling; 1 — standard; 2 — varied Na_o^+ and I_{Na} related to standard; 3 — varied Ca^{2+} and I_{si} related to standard.

Differences in the values of $v_{o,max}$ obtained in physiological experiments between low-Na (50 % NaCl) and low-Ca (25% $CaCl_2$) solutions show that in rabbit pacemaker cells about 20—30 % of I_{si} are carried by Ca^{2+} ions, and 70—80 % by Na^+ ions. In high-Ca solution (200 % $CaCl_2$) the contribution of Ca^{2+} to I_{si} can reach 45 %. Based on the data of computer experiments we could conclude that the contribution of Ca^{2+} ions to I_{si} was about 40 % when the cells were in low-Ca solution (25 % $CaCl_2$). Hence, the contribution of Ca^{2+} released from Ca^{2+} depots was about 20 %.

Thus a comparison of the results of modelling and physiological experiments allows to estimate in the first approximation the contributions of Na^+ and Ca^{2+} ions to the generation process of I_{si}. The model obviously needs further improvement so that kinetic variables of I_{si} would reproduce the respective contributions of ions and their interaction in experimental conditions.

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