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A MODEL OF I_{Kto}-CHANNEL FUNCTION IN RAT VENTRICULAR CARDIOMYOCYTES

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Summary: *The paper presents biophysical characteristics of transient outward potassium current (I_{Kto}) obtained from experiments on isolated rat ventricular cardiomyocytes. Based on these data a quantitative model of rat I_{Kto} was designed.*

1. Introduction

The transient 4-aminopyridine-sensitive outward potassium current, I_{Kto}, is one of the ionic membrane currents involved in repolarization of cardiac cells. It is present in most species including rat, dog and human where it induces a marked lowering of the plateau level of action potential. To simulate the effect of various drugs on this current we designed a mathematical model of I_{Kto} based on electrophysiological measurements on rat ventricular cells. In this paper, we describe the basic characteristic of rat I_{Kto} and their quantitative description.

2. Materials and Methods

Ventricular myocytes were isolated from right ventricular walls of adult male Wistar rats (250 ± 50 g). The dissociation procedure as well as the composition of solutions used in experiments are described in Bébarová et al., (2005).

For measurement of I_{Kto} using patch-clamp technique, only single rod-shaped cells with well visible striations were used. The resistance of the filled glass electrodes was below 1.5 MΩ to keep the access resistance as low as possible. For generation of experimental protocols and data acquisition the Axopatch 200A equipment (Axon Instruments, Inc.) and pCLAMP program (version 6.0.4) were used. The series resistance was compensated up to 80 %. The currents were filtrated at 2 kHz with a four-pole Bessel filter, digitally sampled at 10 kHz and stored on the hard disc. Experiments were performed at room temperature (22 ± 2 °C). Experimental protocols are described in Results. The data are presented as means ± S.E.M from *n* cells. The curve fitting was performed using the software GraphPad Prism, version 4.

The numerical solution of the model was performed using the computational software MATLAB, version 6.5 (The Math Works, Inc.).

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3. Results

Fig.1 illustrates the basic characteristics of rat I_{Kto}. The voltage dependence of time course of I_{Kto} is depicted in Figure 1A. The currents were elicited by 500 ms depolarizing pulses from holding potential -75 mV to +75 mV (with 15 mV increments) applied at 0.2 Hz. Pulses were preceded by 20 ms lasting prepulse to -40 mV to inactivate the rest of sodium current that was not blocked by 20 μmol/l tetrodotoxin. The voltage-dependent changes of the rate of I_{Kto}-inactivation were evaluated from the same experimental protocol. The time constant τ_i increased in an almost linear way with increasing voltage of the imposed pulses between 0 mV and +75 mV (Fig. 1B). The voltage dependence of the steady-state I_{Kto}-inactivation (Fig. 1C) was determined using 500 ms test pulses at +60 mV (applied at 0.2 Hz) preceded by 500 ms conditioning prepulse between -100 mV and 0 mV (with 10 mV increments). The fit of this data by sigmoid function revealed the slope factor of 4.3 ± 0.2 mV and the voltage at half-maximal inactivation of -41.3 ± 0.2 mV.

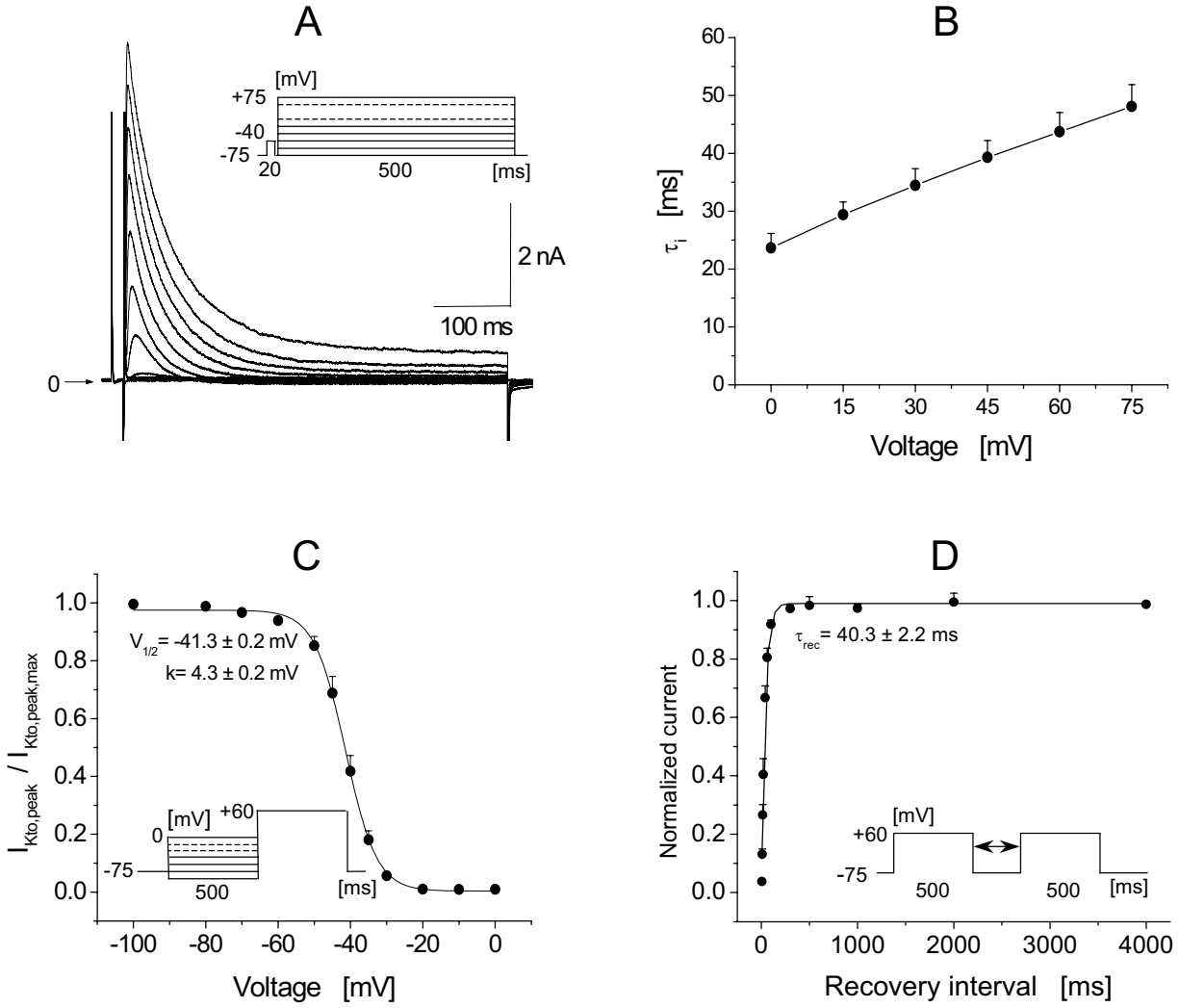

Figure 1. Basic characteristics of rat $I_{Kto.}$ A: Voltage dependence of time course of I_{Kto}. B: Voltage dependence of time constant of I_{Kto}-inactivation. C: Voltage dependence of the steady-state I_{Kto}-inactivation. D: Recovery of I_{Kto} from inactivation. The experimental protocols are depicted in insets.

Recovery of I_{Kto} from inactivation was evaluated by a standard double pulse protocol. The two pulses (from –75 to +60 mV, 500 ms) were separated by a recovery interval that was gradually prolonged from 5 to 4000 ms. The frequency of stimulation was 0.1 Hz. As shown in Fig. 1D, I_{Kto} was completely recovered from inactivation within 300 ms. The time course of the recovery could be well approximated by a single exponential function with the time constant of 40.3 ± 2.2 ms.

The quantitative model of rat I_{Kto} was constructed on the basis of the present experimental data and our previous models. The kinetic scheme of channel gating (Fig. 2) and the rate constants controlling channel activation (α_q, β_q) were adopted from our previous work (Bébarová et al., 2005). However, the description of channel inactivation and recovery from inactivation (rate constants α_r and β_r) was completely reformulated to comply with results in Fig.1.

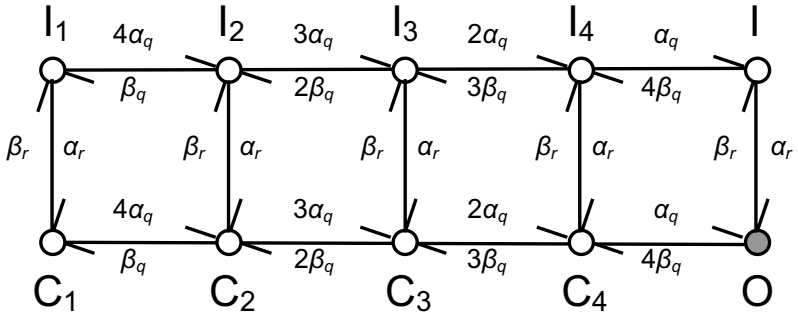

Figure 2. Kinetic scheme of K_{to}-channel gating. The symbols C_x, I_x and O denote closed channel states, inactivated channel states and open channel state, respectively.

The complete set of equations constituting the model of K_{to}-channel gating and the Ohm relation for computation of I_{Kto} is following:

$$\alpha_q = 395/(1+\exp(-0.081\times(V_m+0.9)))$$

$$\beta_q = 356/(1+\exp(0.0463\times(V_m+12.4)))$$

$$\alpha_r = 0.10439\times\exp(-0.07252*V_m)$$

$$\beta_r = 1/(0.0004\cdot\exp(-0.165\times V_m)+0.02436\times\exp(V_m\times 0.00869))$$

$$I_{Kto} = g_{Kto}\times O\times(V_m - E_K).$$

The symbols V_m, E_K, O and g_{Kto} stand for membrane voltage, reversal voltage for K^+ ions, probability of channel opening and conductivity of all K_{to}-channels in cellular membrane, respectively. The units in which the equations were solved were: mV for membrane and reversal voltages, μS for channel conductivity and s^{-1} for rate constants. To comply with the experimental results in Fig.1, E_K was set to -80.8 mV and g_{Kto} to 0.058 μS.

The ability of the model to reconstruct experimental data is demonstrated in Fig. 3. The upper part (Fig. 3A) shows the comparison of time course of I_{to} during depolarizing pulse. The left bottom part (Fig. 3B) illustrates the comparison of steady state inactivation and the

right bottom part (Fig. 3C) illustrates the comparison of the time course of I_{to} recovery from inactivation. As follows from the figure, the model simulations are in very good agreement with results of real experiments.

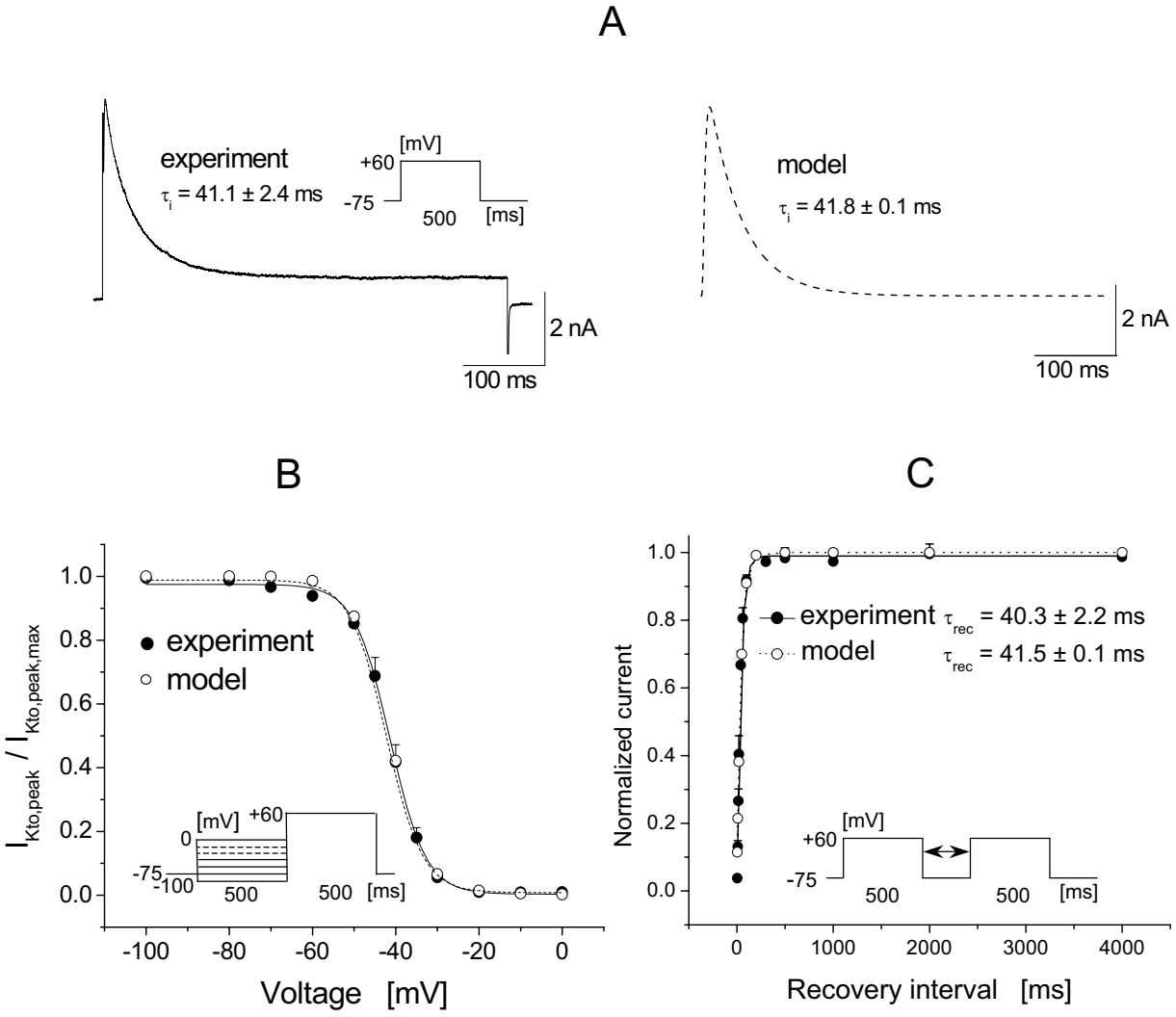

Figure 3. Comparison of experimental and simulated results. A: Time course of I_{Kto} elicited by 500 ms depolarizing pulse from holding potential -75 mV to +60 mV. B: Voltage dependence of the steady-state I_{Kto}-inactivation. C: Recovery of I_{Kto} from inactivation. The experimental protocols are depicted in insets.

4. Conclusion

The biophysical characteristics of rat I_{Kto} obtained in our experiments could be satisfactorily reconstructed using the quantitative model of K_{to}-channel described in this paper. This qualifies the model to be implemented in a complex model of rat ventricular cell electrophysiological activity to simulate the various mechanisms of pharmacologically induced I_{Kto}-block and its physiological consequences.

5. Acknowledgements

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6. References

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