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Capacitative Properties of Cell Membrane as Evaluated in Voltage-Clamped cultured Embryonic Chick Heart Cells

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Abstract Using cultured embryonic chick heart cells, the electrical capacitance of cell membrane (C_m) was measured by square and ramp pulse methods. The C_m Values obtained by both methods are in accordance not only with each other, but also with those calculated theoretically. Using a modified P/4 method, we could successfully eliminate the capacitative currents (I_c) alone, so as to isolate the ionic current. We confirmed that both C_m-measuring and I_c-cancelling procedures employed here were efficient.

Key Words : electrophysiology, membrane capacitance, voltage-clamp, heart cell

Introduction

We have been investigating the modes of action of class-1 antiarrythmic agents in heart. Most of such studies were carried out using V_{max} as a measure of the Na^+[(1,2)]. To analyse antidisrythmic drug actions in further detail, direct measurements of the Na^+ current is required. We recently proposed that the embryonic chick ventricular cells are suitable for this purpose with respect to the better 'space-clamp' under the physiological Na^+ concentration, because of their spheroidal shapes and small cell sizes[(3)]. When we study the detailed kinetic properties of Na^+ channels. however, we have to take factor of the membrane capacitance into account.

The present study focusses on (1) estimations and comparison of C_m values by two different methods, (2) whether or not the C_m values so estimated have rationale, and (3) test of our modified P/4-method[4)] for cancelling the capacitative transients.

Materials and Methods

Single heart cells were prepared according to the method described previously[(3)]. In brief, the hearts from 3- to 17- day-old chick embryos were dissected from fertilized eggs under sterile conditions. Minced ventricular tissues were placed in the Ca/Mg-free phosphate-buffered solution (PBS) containing trypsin (0.1 0.25g/100ml). Myocytes were dispersed by strirring gently for 5 min at 37℃. Two milliliters of the cell suspension (M-199 + 10% new-born calf serum) were seeded into culture dishes, and the culture was placed in a CO_2 incubator at 37℃ for 5 -20 hr.

Incubation for > 5 hr allowed cells to adhere loosely to the bottom of the dishes, enabling a single-cell voltage-clamp (v-c) experiment[(5)] to be done. Under microscopic observation, the cell diameter was visually

Table 1 Summary of Experimental Results

	A	B	C		D	E
	Cell Diameter (μm)	Surface Area (10^{-10} μm^2)	Membrane a. square	Capacitance (pF) b. ramp	Specific Capacitance ($\mu F/cm^2$)	Reversal Potential (mV)
1	13.75	5.94	6.15	6.20	1.04	+62.3
2	11.25	3.98	4.45	4.60	1.12	−53.8
3	13.75	5.94	6.26	6.30	1.05	+65.5
4	15.00	7.07	7.60	7.65	1.07	+65.0
5	8.75	2.41	2.35	2.05	0.98	+45.0
6	13.75	5.94	7.00	7.06	1.18	+61.0
7	17.50	9.62	12.00	12.13	1.25	+70.3
8	11.25	3.98	4.24	4.20	1.07	+62.0
9	12.50	4.91	5.70	5.75	1.17	+58.4
10	15.00	7.07	7.76	7.80	1.10	+65.3
N=10	13.25± 0.81	5.67± 0.67	6.35± 0.86	6.37± 0.89	1.10± 0.03	+60.86± 2.38

Surface area (B) was calculated by πx D^2, D is the diameter of spheroidal cells (in μm, given in A). Values for the membrane capacitance (C, in pF) were obtained by both the square- (a) and ramp (b)-pulse methods. The specific capacitance (C_m value normalized with respect to the membrane area) is the value of the membrane capacitance (shown in C-a) divided by the surface area (B). Values on the bottom; mean ±S.E.

scaled (Table 1 A). Cells chosen for v-c study had a following criteria: (1) Shape, sphere or spheroidal; (2) diameter, $< 15\mu m$; (3) Single cells, being isolated from surrounding cells. Experiments were carried out at 20℃.

To determine C_m, two methods (square- and ramp-pulse methods) were employed. After establishing the patched-membrane seal, the capacitative current derived from the glass wall (C_{glass}) was manually minimized using internal compensation circuitry of an amplifier (EPC-7) until obtaining the smallest size of capacitative surge . The residual capacitative currents associated with C_{glass} (usually 1-2 pF) were stored, as both 10 mV-square steps (square-pulse method), and, subsequently, the ramp pulses (ramp-pulse method) were given. After rupture of the patched membrane, currents derived from the cell membrane capacitance (C_m) added further to $I_{c(glass)}$ on the current signal. Subtraction of $I_{c(glass)}$ left the C_m -related I_c alone.

In the square-pulse method, the area [A] under the capacitative spike was calculated. The area reflects the quantity of charge movements [q] , which is equal to C_m X V. Hence, the C_m value can be deduced from the equation: $C_m = A/V$.

In the ramp-pulse method, the amplitude (in ampere) of I_c in response to ramp-pulses (slope; 5 V/sec) was measured. As capacitative current [I_c] flows according to the equation: $I_c = C_m\, dV/dt$, a constant I_c is expected when ramp-pulses having a constant dV/dt are given. The value of C_m was calculated from the equation: $C_m = I_c/5$.

To cancel the capacitative transient in the v-c study, a modified P/4-method was devised. With the aid of a computer, pulses of varying amplitudes and arbitrary prepared as the main pulse, concomitantly with inverse pulses having 1/4 amplitude of the main pulses (inverse P/4-pulse) so prepared were delivered to cell via a D/A converter (Shosin EM, OI-8). By appling each main pulse with succsessive four inverse P/4 -pulses together, the capacitative current could be subtracted. Ionic currents *per se* here were not affected by these P/4-pulses.

For the fast Na^+ current recording, K^+ ions in both the bath and pipette solutions were substituted Cs^+ ions. In addition, the pipette solution contained 20 mM TEA Cl.

Results

Values of C_m obtained by square- and ramp-pulse methods are presented in Table 1 (C, D). C_m values calculated by both methods coincided with each other. In the table, values of the specific C_m, C_m normalized with respect to the surface area of cell membrane, also are listed. In any measurement, the specific C_m values are found to be close to 1 μF/ cm^2.

For I_c-cancellation, we tried a modified P/ 4-method. The effect of I_c elimination was obvious when the v-c data were presented in terms of the current-voltage relation. The difference in Na^+ current values before and after I_c-cancellation was large at potentials 0 mV to c. a. +70 mV, over which I_{Na} magnitude became smaller for more positive potentials while the I_c magnitude increased.

As a result, the reversal potential (V_{rev}), the potential at which the current altered its sign from negative to positive, was less positive before, an more positive after I_c-cancellation. The extent of the shifts in V_{rev} toward the positive values ranged between 10~30 mV in 10 experiments. Thus, the effect of cancellation can be confirmed, employing V_{rev} after I_c subtraction (Table 1, E). As shown here, the values of V_{rev} after cancellation are close to the Na^+ equilibrium potential (E_{Na}) of +66.7 mV.

Discussion

Since the lipid bilayer in a cell membrane is an electrical non-conductor and since the elctromotive force is present between inside and outside of the membrane, the lipid bilayer functions as a capacitor. Ion-permeating pores in proteins embedded in the bilayer can work as variable resistors. In total, the cell membrane *per se* is regarded as an electical circuit in which both capacitors and resistors are installed in a parallel combination. During the cardiac action potential (AP), the membrane undergoes the discharging/charging sequence, as described by the equation: $dV/dt = -1 / C_m \cdot I_{ion}$, where dV/dt, C_m, and I_{ion} denote the first time-derivative of voltage (i. e. AP), membrane capacitance, and ionic currents, respectively. Hence, the determination of the exact value of C_m is essential when we reproduce the time course of AP based on I_{ion} data. In this sense, C_m, electrophysiologically, is a very important parameter.

In contrast, the cell membrane capacitance is one of obstacles for voltage-clamping the cell membrane. The large capacitative currents always overlap the ionic currents so as to distort the current records. Consequently, not only must we establish a method for cancelling the capacitative currents, but also we must know the exact value of the cell membrane capacitance.

For determining C_m we tried 2 methods, and compared them. Calculations by both methods gave C_m values similar to each other. In general, the capacitance (C) depends on: (1) the area [A] of the conductor plates; (2) the dielectric properties of the insulating materials which space the plates (ε); and (3) the distance [l] between plates[6]. The capacitance [C], in turn, is given by the equation: $C = A \times \varepsilon_0 \times \varepsilon_s / l$. In this equation ε_0 is the dielectric constant for air (c.a. 8.842 x 10^{-12} F/m), and ε_s is 6 for the lipid. Assuming that in v-c experiments the width of the non-conductor (i.e. bilayer) is 5 nm and that the diameter of spheroidal cell is D μm (i.e. $A = \pi \times D^2 \times 10^{-12}$ m^2), then the above equation can be written: $C_m = 0.033 \times D^2$ pF. For D=10~15 μm as in our study, the equation gives 3 pF~7.5 pF as C_m. This value is comparable to ours, indicating that C_m was satisfactorily determined by our methods.

The specific capacitance value averaged 1. 1 $\mu F/cm^2$, a little larger than that previously reported (1 $\mu F/cm^2$). This might attributed, in part, to the procedure for scaling the cell diameter. Alternatively, this might be due to folding of the cultured cell membrane.

We modified the P/4-method that has so far been employed by others[7]. Through this treatment, net ionic current could be now isolated without any hindrance by capacitative currents. As a result, the reversal potential was near the E_{Na} value which

was calculated from the equation (at 20℃):

$E_{Na} = RT/F \cdot \ln [Na^+]_o / [Na^+]_i = 58.17 mV \times \log [Na^+]_o / [Na^+]_i$. In the present experimental conditions ($[Na^+]_o = 140$ mM vs. $[Na^+]_i = 10$ mM), the equation yielded an E_{Na} of +66.7 mV. The most advantageous feature of our modified P/4-method that this is applicable to complicated pulse patterns which are required for studies on Na^+ channel kinetics.

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