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VOLTAGE-ACTIVATED K CHANNELS IN EMBRYONIC CHICK HEART

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Voltage clamp experiments indicate the presence of a time-dependent potassium current activated at potentials positive to −50 mV and involved in repolarization of the action potential of chick embryonic ventricle (1). This potassium current, called I_X, is comparable to the delayed rectifier in Purkinje fibers (I_{X1}, reference 2), adult ventricle (I_K, reference 3), and SA node (I_K, reference 4). In each of these preparations the current we will call I_K is activated in the range between −50 and +10 mV, is time dependent with a time constant in the range of hundreds of milliseconds, and is an inward rectifier. The reversal potential reported for I_X is variable. In Purkinje fibers it was found to reverse at −65 mV in 4 mM K_O and was thus considered not completely specific for K ions (2). In ventricle, however, the delayed rectifier reversed at −90 mV in 3 mM K_O (3). The delayed rectifier in nerve has been shown to have a single channel conductance in the range of 12–17 pS (5, 6). There are few data on single channels in heart cell membrane to compare with macroscopic data on the delayed rectifier. We report single channel measurements on a current in 10-d chick embryonic ventricle that is activated in the same range as I_X, reverses at −88 mV in 4 mM K_O, and appears to rectify inwardly above 0 mV.

RESULTS

Although we have not measured the relative ion selectivity of the channel, Figs. 1 and 2 indicate that it is highly selective for K. First, the reversal potential for the current is near the K equilibrium potential. For 140/4 mM K gradient in the cell-attached experiments (Fig. 1 *a–c*), the Nernst potential at room temperature is −90 mV. The measured reversal potential is −87 mV (Fig. 2). Second, in outside-out patches where the K gradient is controlled (Fig. 1 *d*), the channel has the expected conductance and kinetics if the data are shifted along the voltage axis by the K Nernst potential.

The open state of this channel shows the phenomenon known as flickering, that is, the open state appears to occur in groups separated by closed times that are long compared to those within a group. It is evident from Fig. 1 that the mean length of these groups varies from tens of milliseconds at −30 mV to hundreds of milliseconds at 20 mV. Although the fraction of time spent in the open state increases dramatically as the membrane is depolarized, the length of time the channel actually stays open is rather brief. Open time histograms fit one exponential but closed time histograms are the sum of at least two exponentials (see legend, Fig. 3).

The open channel $i(V)$ relationship shown in Fig. 2 *a* appears to rectify inwardly at large positive voltages. It is unclear at the present time whether this is a real effect due to open channel conductance or whether this apparent depression of the currents at higher voltages results from increased flickering. The $i(V)$ data fit extremely well between −70 and 0 mV to a simple ohmic relationship given by $i = \gamma (V-E)$ where $\gamma = 62$ pS and $E = -87$ mV. A quadratic curve that fits all the points better than a straight line is given in Fig. 2 for comparison. The apparent curvature of the $i(V)$ data is not due to a varying patch-seal resistance. This is shown in Fig. 2 *b*, where the background current through the patch-seal resistance is measured. The background current is the current that flows through the parallel combination of the seal resistance and the patch membrane when the channel is closed. These points are fit to a quadratic function of voltage for comparison with Fig. 2 *a*, but they essentially lie on a straight line with a slope of 41 pS or 24 GΩ.

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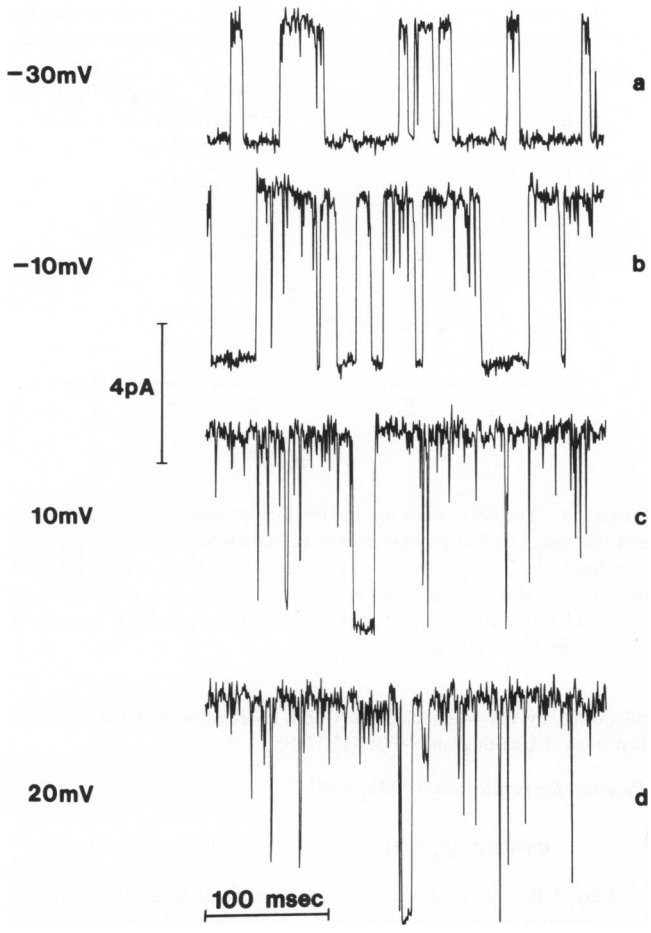

FIGURE 1 Single-channel records from 10-d chick embryo ventricle cells prepared as clusters 20–40 μm in diameter and maintained in tissue culture. All experiments were done at 27°C in a balanced salt solution containing 4.0 mM K and 1.8 mM Ca in the bath. A similar solution was used in the pipette but with zero Ca. Under these conditions there is a stable resting potential of -49 ± 3.3 mV as determined in separate microelectrode experiments. Records *a–c* are from a cell-attached preparation with the pipette potential clamped at -20 mV, -40 mV and -60 mV; record *d* is from an outside-out patch with the pipette clamped at $+20$ mV. In *d* the pipette contained 150 mM K. The absolute transmembrane potentials across the patch are -30 mV, -10 mV, and 20 mV, as shown in the figure. An upward deflection represents the opening of a channel carrying an outward current. As the patch is depolarized, the current through the open channel increases and the channel spends more time in the open state. Only the steady-state records are analyzed, selected 0.5 s after the beginning of a 4-s voltage clamp step from the resting potential. The open state is interrupted by numerous brief closings, called flickering.

The open channel probability is a sigmoidal function of voltage that increases as the channel is depolarized. The expression used to fit the data is $p(V) = 1/(1 + \exp[aV + b])$ where, from Fig. 3, $a = -0.0653$ and $b = -0.871$.

The experimental points were obtained without regard to the phenomenon of flickering. Note, however, that although $p(V)$ approaches unity at large positive voltages, the length of time the channel actually stays open has a mean value of <17 ms.

The expected macroscopic steady-state current from a

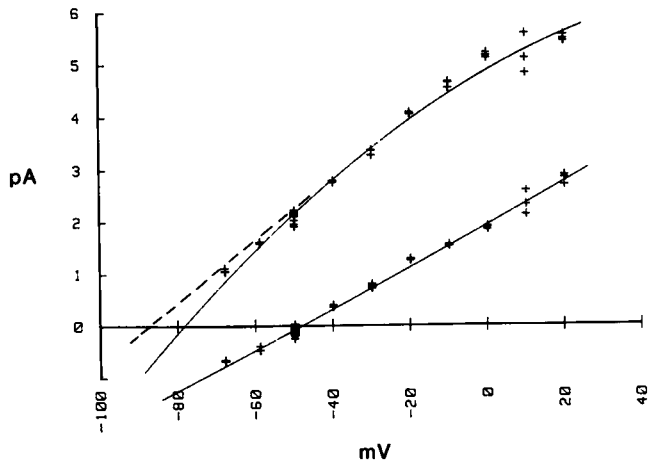

FIGURE 2 *a*, the open-channel $i(V)$ curve constructed from amplitude histograms of single-channel records. Each point represents the difference between the means of two Gaussian curves fit to the open and the closed state of the channel. *b* shows the mean current through the seal-patch resistance when the channel is closed. The solid line in *a* is a least-squares fit to the quadratic equation $I(V) = c + bV + aV^2$ where $c = 4.87$, $b = 0.0416$, and $a = -2.65 \times 10^{-4}$. From this equation, channel conductance is 68 pS at -50 mV and the reversal potential is -77 mV. The dashed line is a least-squares fit to a straight line excluding the data positive to 0 mV. Using this line, the channel conductance is 62 pS and the channel reversal potential is -87 mV. See text for discussion. The solid line in *b* is a least-squares fit to a quadratic equation with $c = 1.90$, $b = 0.0415$, and $a = 1.90 \times 10^{-5}$. The straight-line fit to these data is virtually indistinguishable from the quadratic fit, and gives a closed channel patch-seal conductance of 41 pS and a reversal potential of -47 mV.

population of N such channels is $I = Nip$. This relationship is shown in Fig. 4 for $N = 1$. If we assume the value of I_X (-30 mV) = 1.5 μA/cm^2 from Clay and Shrier (1),[1] the estimated channel density for the delayed rectifier in embryonic chick ventricle is two channels/100 μm^2.

DISCUSSION

Macroscopic experiments on the delayed rectifier in heart show two time constants, one the order of seconds and the other the order of 100 ms. In some preparations the slower of these two time constants is thought to be due to ion accumulation in the interstitial space; however, there is evidence that it may be due to channel kinetics. Voltage-dependent time constants in the 100-ms range do appear in our single channel records. These do not result from long open times but from clusters of much faster transitions open-close transitions separated by relatively long closed times. This millisecond component of channel kinetics for the delayed rectifier does not appear in macroscopic experiments on cardiac membranes, probably for technical reasons. It is, of course, observed in the analogous K current in nerve when the action potentials are 100 times briefer than in heart. At the present time there are few single-channel data on K currents in nerve with which to compare our results. We note, however, that the delayed-

[1]Clay, J. R., and A. Shrier. Personal communication.

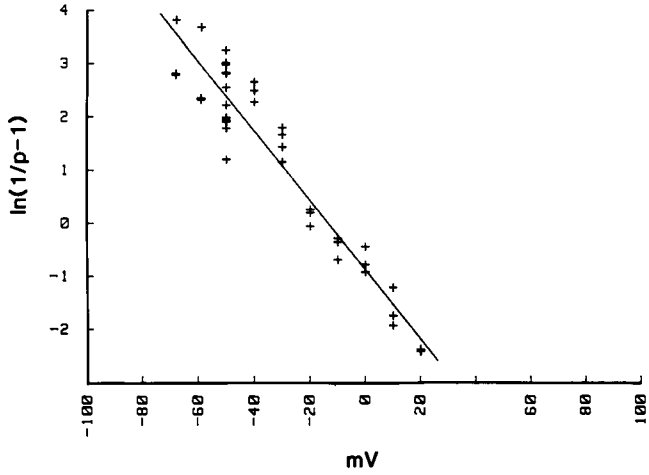

FIGURE 3 The dependence of the steady-state open channel probability (p) on membrane potential (V). The points are measured as follows: the amplitude histogram of raw single-channel records is fit by two Gaussians centered on the mean open-state current and the mean closed-state current. An event is defined as data points that fall outside 2.56 SD. The time the event occurs is found by extrapolating these points back to the mean. For partial openings (or closings) that do not reach the closed (or open) state, the duration of the event is taken at the moment the data set comprising the event reaches its largest value, or at the moment the data set first reverses the sign of its slope. In this way, an idealized sequence of openings and closings is obtained from the raw data. The points are 0.1 ms apart for data taken at 1,000 Hz bandwidth. The steady-state open-channel probability is the fraction of time spent in the open state between 0.5 and 4.0 s after the voltage step. The figure tests the theoretical curve $p(V) = 1/1 + \exp(aV + b)$ by comparing the data points to the solid line $\ln(1/p - 1) = aV + b$ where $a = -0.0653$ and $b = -0.871$. The single time constant from open time histograms depends on voltage according to $\tau_0 = 11 \exp(V/46)$ ms. The slow component of the closed times obeys τ_c (slow) $= 9.2 \exp(-V/22)$ ms. The fast component of the closed times is 13.6 ms at -50 mV and decreases to < 0.4 ms above -20 mV.

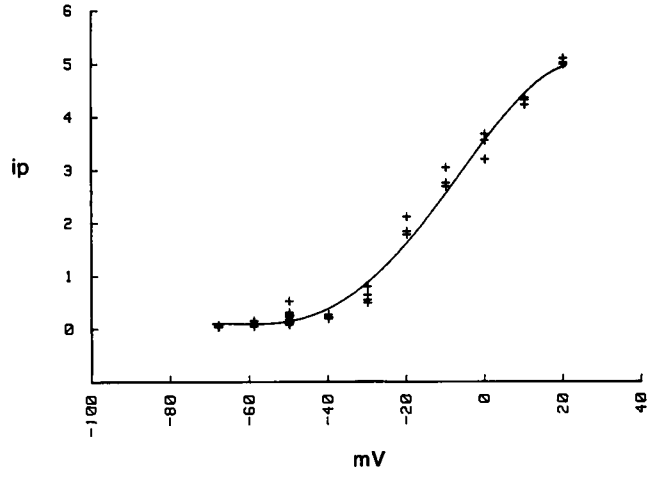

FIGURE 4 The data points are the product of open-channel current (i) and the open-channel probability (p) vs. membrane potential (V). The solid line is the theoretical $i(V) \cdot p(V)$ relationship, where $i(V)$ is the solid line fit to the data in Fig. 2 and $p(V)$ is the theoretical curve taken from Fig. 3. This product is proportional to the macroscopic steady-state $I(V)$ relationship for this current.

rectifier single-channel conductance in heart is four times larger than the K channel conductance found in nerve (5, 6), but the channel density in heart estimated here is >1,000 times lower than in nerve (5). This conclusion depends, of course, on a literal interpretation of the macroscopic data but it seems inescapable that the density of the delayed rectifier channel in heart is extraordinarily low.

Supported by the Massachusetts Heart Association and the National Institutes of Health grant 1-P01-HL27385.

Received for publication 12 May 1983.

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SIZE-DEPENDENT KINETICS ASSOCIATED WITH DRUG BLOCK OF SODIUM CURRENT

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BASIC MEASUREMENT

Sodium channel block by many drugs is modulated by rate of use of the channel. For instance, rapid trains of action potentials or depolarizing pulses will enhance channel block to above the basal (resting) level of block. After such conditioning trains, channel block relaxes back to the basal (b) level with widely varying time constants (T). Sodium

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Volume 45 January 1984 42–44