A NOTE ON THE REACTIVATION OF THE FAST SODIUM CURRENT IN SPHERICAL CLUSTERS OF EMBRYONIC CHICK HEART CELLS

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ABSTRACT Reactivation of the fast sodium current in spherical clusters of embryonic chick heart cells was studied using the two-microelectrode voltage-clamp technique. The results show that there are at least two phases of reactivation. The contribution of the two phases to the overall reactivation process is highly dependent on the particular pulse protocol used to measure them.

INTRODUCTION

One reputed difference between the fast sodium permeability of cardiac muscle and that of squid axon is that in cardiac muscle the time course of reactivation of the fast sodium current is one to two orders of magnitude slower than the time course of inactivation at the same membrane potential. Haas et al. (1971) reported that in frog atria, using the sucrose-gap voltage-clamp technique, both recovery from inactivation following a depolarizing voltage-clamp pulse and reactivation following application of a hyperpolarizing pulse had time constants of 100–600 ms at 4–7°C. Comparison of the time constant of inactivation as determined by a single-pulse method (τ_h) with those of reactivation (τ_r) at the same potential gave typical ratios of 1:50. A slow recovery from inactivation has also been reported by Gettes and Reuter (1974) in ventricular fibers from sheep, calf, and pig hearts and more recently by Brown et al. (1980, 1981) in isolated perfused rat myocytes. In the earlier report, Brown et al., (1980) found the time constant of reactivation to be eight to ten times larger than that of inactivation as measured by a single- or double-pulse method. In contrast, Ebihara et al. (1980) reported that in cultured clusters of embryonic chick heart cells, the time constant of inactivation and that of reactivation at the same potential were similar, and that the processes of activation, inactivation, and reactivation were satisfactorily described by the Hodgkin-Huxley formulation (Ebihara and Johnson, 1980).

The purpose of the present communication is solely to draw attention to the likelihood that this discrepancy in the behavior of chick and other cardiac muscle stems, at least in part, from differences in the experimental protocols employed and not from fundamental differences in the kinetics of the fast current.

METHODS

The preparation of small spherical aggregates (50–90-μm diam) of tissue-cultured embryonic chick heart cells together with details of the two-microelectrode voltage-clamp technique have been described previously (Ebihara et al., 1980). As in that previous study, all experiments were conducted at 37°C in a bicarbonate-CO_2 buffered culture medium; the data were obtained within the first 6 min after successful penetration with the microelectrodes. We used the first 3 min to adjust parameters of the pulse sequence and the remainder to execute the series of pulse sequences; each pulse sequence was separated from the next by 700 ms during which time the membrane potential was held at −60 mV.

RESULTS

Reactivation of the fast sodium current was first studied by applying a hyperpolarizing conditioning prepulse of variable duration from a holding potential of −60 mV followed by a test pulse to −20 mV. The peak current during the test pulse, I_p, was then plotted as a function of the duration of the conditioning prepulse. Fig. 1 shows reactivation curves from a typical preparation for potentials ranging between −80 and −65 mV. The solid lines represent the best fit of a single exponential to the data. The time course of reactivation was adequately described by a single exponential in all experiments, as reported previously (Ebihara, et al., 1980; Ebihara and Johnson, 1980). Fig. 2 compares

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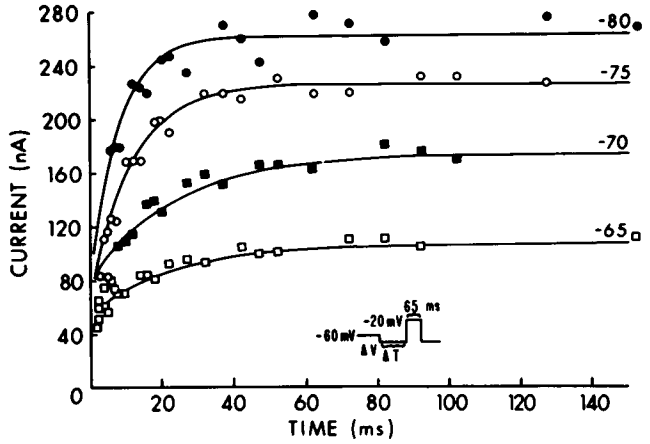

FIGURE 1 Peak I_{Na} during a test pulse to −20 mV is plotted against the duration of a conditioning prepulse to the potential indicated above each curve. The holding potential is −60 mV. The solid lines are single exponentials; the symbols are experimental results.

the time constants of such reactivation with those of inactivation over the same potential range obtained with a double-pulse method. No significant difference was observed.

The time course of recovery from inactivation induced at more positive potentials was also studied, using the double-pulse method of Hodgkin and Huxley (1952). Two depolarizing pulses were applied in succession from a holding potential of −60 mV. The time interval between the two pulses was varied, and the peak current in response to the second pulse was measured as a fraction of that in response to the first. Provided that the duration of the first pulse was <50 ms, the time course of recovery was satisfactorily described by a single exponential. On the other hand, for longer durations, the time course of recovery from inactivation was better described by two exponentials. Fig. 3 illustrates this effect. Following short depolarizing prepulses, the fast current recovered exponentially with a time constant similar to that of inactivation at the same potential. When the duration of the prepulse was increased, however, the fast current began to recover in two phases, an initial fast and a subsequent slow phase; the magnitude of the slow phase relative to the fast phase increased as the duration of the prepulse was increased; the time constant of each phase remained essentially unchanged. Similar results were obtained in five other experiments. (The variation in the time constant of the fast and slow components with duration of the prepulse, as seen in Fig. 3 and in the fits to data from other experiments, was not considered significant; the variation was inconsistent and could be attributed to the scatter in the experimental points.) To exclude the possibility that inactivation and reactivation of an accompanying slow inward current was the underlying factor responsible for these observations, the above experiment was repeated in a preparation exposed to D600, a substance that has been shown to eliminate the slow inward current in preparations such as those used in this communication (Ebihara et al., 1980). Similar results were obtained.

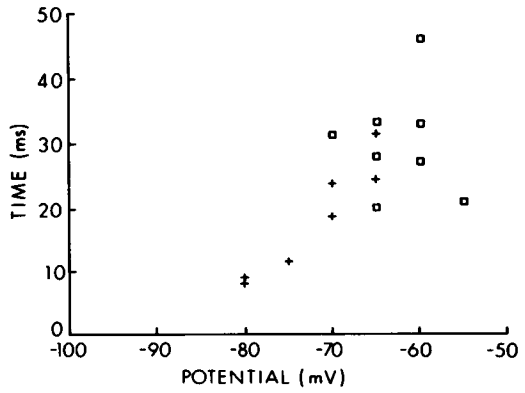

FIGURE 2 Time constants of inactivation (τ_c) and reactivation (τ_r) obtained by a two-step method plotted as a function of membrane potential. □, τ_c; +, τ_r.

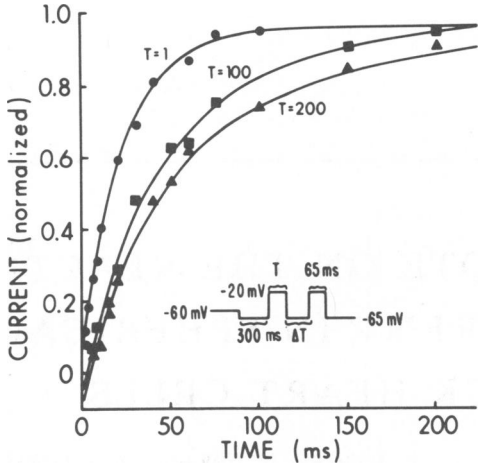

FIGURE 3 The time course of reactivation following an inactivating prepulse to −20 mV of three different durations (T) as indicated beside each curve. The sequence of membrane potential changes is shown in the inset. *Abscissa:* Time after the end of the prepulse when the test pulse was applied (ΔT in inset); *ordinate:* I_t/I_0 in which I_t is the peak current during the test pulse and I_0 is the peak current during the prepulse. Experimental results represented by ● (T = 1 ms), ■ (T = 100 ms) and ▲ (T = 200 ms). The solid lines are double exponential fits of the data of the form $A \cdot \exp(t/\tau_1) + B \cdot \exp(t/\tau_2)$, minimizing the least-square function using PRAXIS, an algorithm for finding zeros and extrema of functions without calculating derivatives (Brent, 1973). For a depolarizing prepulse (T) of 1 ms, $A = 0.92$, $\tau_1 = 24$ ms, $B = 0.035$, $\tau_2 = 100{,}000$ ms (i.e., the data are fitted with the single exponential term, $A \cdot \exp[t/\tau_1]$). For 100 ms, $A = 0.60$, $\tau_1 = 32$ ms, $B = 0.47$, $\tau_2 = 85$ ms. For 200 ms, $A = 0.47$, $\tau_1 = 23$ ms, $B = 0.67$, $\tau_2 = 100$ ms.

DISCUSSION

In embryonic chick heart cells there appear to be at least two phases in the reactivation of the fast inward current. The faster phase represents recovery from a fast, *h*-in-

activation process (see Ebihara and Johnson, 1980), the time constant of recovery for this process being approximately equal to the time constant of inactivation at the same potential. The second, slower process may represent the recovery from a second inactivation process for the sodium current such as that reported in *Myxicola* axon (Schauf, et al., 1976), frog node of Ranvier (Peganov, et al., 1973; Fox, 1976), squid giant axon (Narahashi, 1964; Adelman and Palti, 1969*a, b;* Chandler and Meves, 1970), and more recently in single isolated rat heart cells (Brown et al., 1981). One consequence of the present findings is that the observed reactivation time constant is highly dependent on the pulse protocol used to measure it. When the time course of reactivation was studied by a hyperpolarizing pulse of variable duration from a holding potential of −60 mV, only the faster process is observed. On the other hand, when reactivation is studied by first applying a depolarizing prepulse to inactivate the fast sodium current more completely, two phases of reactivation were seen, depending on the duration of the depolarizing prepulse. For sufficiently brief prepulses, only the faster process was observed, whereas for longer-lasting (≃200 ms) prepulses the slower process predominated. Conceivably, the slower process could be invoked by the prolonged depolarization associated with an action potential, especially in those tissues where the action potentials exceed ≃200 ms. In the cultured-cluster preparations used here, however, the time course of recovery of the fast sodium current following an action potential (≃150–200 ms in duration) was fitted by a single exponential, the time constant of which was close to that predicted from voltage-clamp data (Ebihara, et al., 1980).

Thus, depending on the pulse protocol used, it was possible to measure a time constant of reactivation that was equal to, or much longer than the time constant of inactivation measured at the same potential. It would be interesting to see whether a similar circumstance is obtained in other kinds of cardiac muscle where a large difference in τ_h and τ_r at the same membrane potential has been reported (Haas et al., 1971; Brown et al., 1980).

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