SLOW INACTIVATION OF A TETRODOTOXIN-SENSITIVE CURRENT IN CANINE CARDIAC PURKINJE FIBERS

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ABSTRACT We used the two-microelectrode voltage clamp technique and tetrodotoxin (TTX) to investigate the possible occurrence of slow inactivation of sodium channels in canine cardiac Purkinje fibers under physiologic conditions. The increase in net outward current during prolonged (5–20 s) step depolarizations (range −70 to + 5 mV) following the application of TTX is time dependent, being maximal immediately following depolarization, and declining thereafter towards a steady value. To eliminate the possibility that this time-dependent current was due to inadequate voltage control of these multicellular preparations early during square clamp pulses, we also used slowly depolarizing voltage clamp ramps (range 5–100 mV/s) to ensure control of membrane potential. TTX-sensitive current also was observed with these voltage ramps; the time dependence of this current was demonstrated by the reduction of the peak current magnitude as the ramp speed was reduced. Reducing the holding potential within the voltage range of sodium channel inactivation also decreased the TTX-sensitive current observed with identical speed ramps. These results suggest that the TTX-sensitive time-dependent current is a direct measure of slow inactivation of canine cardiac sodium channels. This current may play an important role in modulating the action potential duration.

The ability to resolve the inactivation and reactivation kinetics of cardiac sodium channels has been limited by difficulties inherent in voltage clamping the fast inward sodium current of multicellular cardiac preparations under physiologic conditions (Johnson and Lieberman, 1971). Recent investigations employing newly developed cardiac preparations and techniques have produced inconsistent results regarding sodium channel kinetics. Sodium channel inactivation in rabbit Purkinje fibers bathed in low Na_o solutions (Colatsky, 1980) and in cultured rat myoballs (Ebihara and Johnson, 1980) has been characterized as a rapid (τ = 1–2 ms) monoexponential process. In contrast, dissociated, internally perfused rat myocytes demonstrate a fast and slow phase of sodium channel inactivation (Brown et al., 1981; Zilberter et al., 1982). Knowledge of cardiac sodium channel inactivation would prove valuable in understanding possible differences between nerve and cardiac sodium channels as well as the genesis of the cardiac action potential.

Tetrodotoxin (TTX), as well as many local anesthetic-type antiarrhythmic agents, shortens the cardiac action potential (Dudel et al., 1967, Davis and Temte, 1969; Gliklich and Hoffman, 1978; Coraboeuf et al., 1979). This shortening is postulated to occur via a reduction of the TTX-sensitive "window" current through sodium channels (Gadsby and Cranefield, 1977; Attwell et al., 1979; Colatsky and Gadsby, 1980; Carmeliet and Saikawa, 1982; Colatsky, 1982). We investigated the possible time dependence of the TTX-sensitive window current to evaluate the presence of slow inactivation of cardiac sodium channels under physiologic conditions.

Shortened Purkinje strands (<2 mm) were voltage clamped using a two-microelectrode voltage clamp technique (Deck et al., 1964; Cohen et al., 1983). Preparations were superfused with Tyrode's solution (bubbled with 95% O_2/5% CO_2) containing (in millmoles per liter) NaCl, 140; $NaHCO_3$, 12; NaH_2PO_4, 0.4; $MgCl_2$, 2.0; dextrose, 8.3; KCl, 5.0; $CaCl_2$, 4.0. Bath temperature was 35°–37°C, and was maintained within 0.5°C in any individual experiment. TTX was obtained from Sigma Chemical Co., St. Louis, MO. Records were recorded with an FM tape recorder (Hewlett-Packard Co., Palo Alto, CA, model 3964A, 15/16 ips [inches per second], 300 Hz bandwidth) for later redisplay and analysis. Membrane currents were compared by computer subtraction of digitized records (12-bit resolution), as well as by visual projection and measurement.

Fig. 1 illustrates membrane currents recorded during three different step depolarizations from a holding potential of −80 mV before, during, and after exposure to 1×10^{-5} M TTX. The difference currents, I_{diff}, obtained by subtracting membrane currents during drug-free control conditions from currents following equilibration with TTX, are displayed in the bottom row. The difference current is not constant, but progressively decreases during each depolarizing test pulse. Qualitatively similar results were obtained in all five similar experiments.

The presumed selectivity of TTX action for sodium channels (Narahashi et al., 1964; Takata et al., 1966;

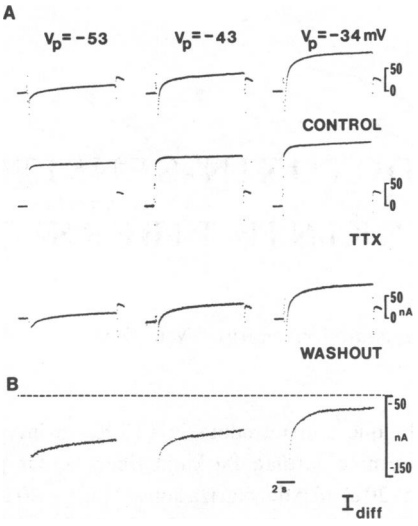

FIGURE 1 Time dependence of the TTX-sensitive difference current. *A*, membrane currents recorded during 10-s depolarizing step pulses (V_p) to −53 (*left*), −43 (*middle*), and −34 mV (*right*) from a holding potential of −80 mV. *B* shows the difference between membrane currents recorded for the various depolarizing steps in the absence and presence of TTX (I_{diff}). Note the different current calibrations in *A* and *B*.

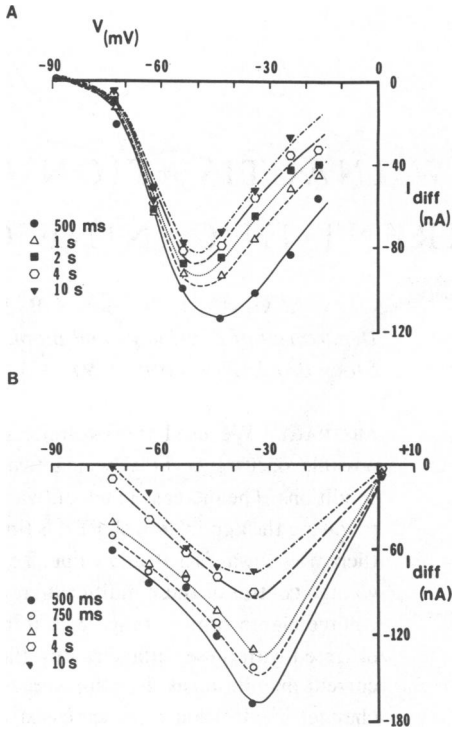

FIGURE 2 Further characterization of the TTX-sensitive difference current. Difference currents obtained during step depolarizations to different potentials are plotted for selected times following the depolarizing pulse onset. *A*, data from the same experiment as in Fig. 1; *B*, results from a different experiment. Isochronal curves drawn by eye without any theoretical basis. Holding potential for *B* was −82 mV. [TTX] = 2×10^{-5} M in *B*.

Hille, 1968; Romey and Lazdunski, 1982) suggests that this time-dependent TTX-sensitive difference current is due to block of a slowly inactivating sodium current. Further evidence for this hypothesis is provided by Fig. 2, where the difference currents are plotted for selected times during 10-s depolarizing pulses to different test potentials in two representative experiments. The isochronal plots illustrate the decreasing magnitude of the difference currents during depolarizing square pulses. The similar configurations of these isochronal plots to those obtained for the steady state TTX-sensitive difference current (cf. Attwell et al., 1979; Colatsky and Gadsby, 1980) further suggest that the time-dependent difference current is a dynamic component of the TTX-sensitive window current.

In these multicellular preparations the membrane potential is not adequately clamped during the first few milliseconds of a depolarizing test pulse due to activation of the fast inward sodium current. This lack of voltage control is reduced or absent in the presence of TTX. We considered it possible that these variations of voltage control, by affecting other time-dependent currents, could have been responsible for the time-dependent difference current observed later during test pulses. To eliminate this possibility, we applied depolarizing ramps to ensure controlled depolarization. Ramp speeds were varied (range 5–100 mV/s) in the absence and presence of TTX to test for a time-dependent current: a decrease in the amplitude of the difference current at any given potential for ramps of slower speeds would demonstrate the existence of a time-dependent TTX-sensitive current that is not contaminated by inadequate voltage control.

A typical example of difference currents obtained with depolarizing ramps is illustrated in Fig. 3. In panel *A*, membrane currents in the absence (lower traces) and presence (upper traces) of TTX have been superimposed on their corresponding ramps with slopes of 35, 17, and 9 mV/s (left to right). An increase of net outward current in the presence of TTX is evident for all three ramp speeds. In panel *B*, the three difference currents have been superimposed such that they are aligned for the same ramp potentials. The difference current is greater with more rapid ramps at potentials where the "window" is expected to be open. Qualitatively similar results were obtained in five additional experiments: three in the absence and two in the presence of CsCl (10 mM) added to block pacemaker currents and reduce background potassium permeability (Isenberg, 1976; Vereecke et al., 1980).

Further evidence that sodium channels regulate this time-dependent difference current is provided by experiments which examined the effect of holding potential on the amplitude of the difference currents obtained with depolarizing ramps (Fig. 4). The peak amplitude of the difference current obtained with depolarizing ramps increases when the holding potential is made increasingly more negative over the range of potentials encompassing the cardiac sodium channel inactivation gating variable.

Numerous studies in nerve preparations under a variety

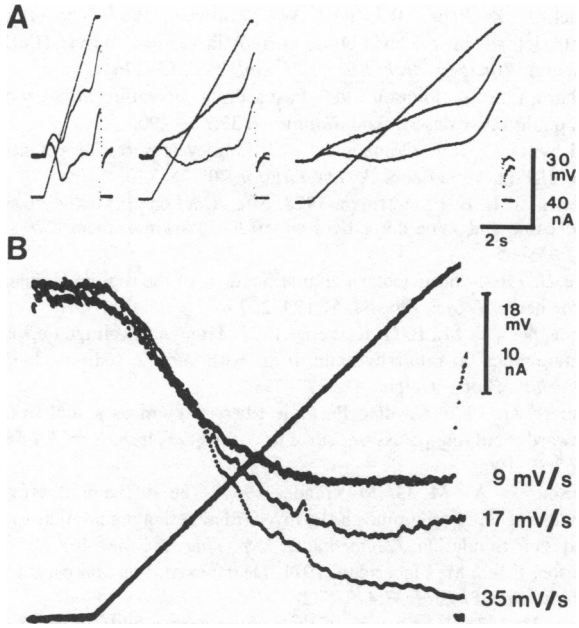

FIGURE 3 A demonstration of the time-dependent difference current with depolarizing ramps. Membrane currents recorded in the absence and presence of TTX for three different ramp speeds (*A*) have been subtracted and superimposed on a single ramp (*B*) such that the difference currents are aligned for any given ramp potential. Ramps of increasing slopes were associated with difference currents of increasing amplitude. Membrane currents recorded after TTX were similar to control records and were eliminated for clarity. Zero current for both panels indicated by top of current calibration. Holding potential = −78 mV. [TTX] = 1×10^{-5} M.

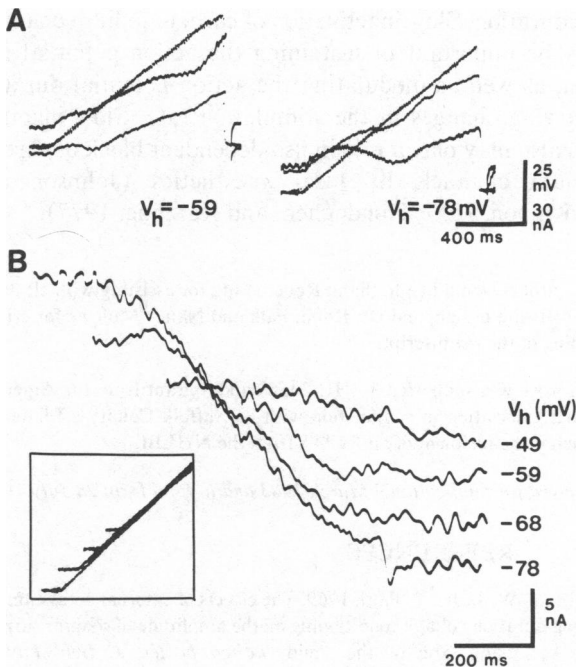

FIGURE 4 The effect of holding potential on the difference current during ramp depolarizations. *A*, membrane currents in the absence (*lower* traces) and presence (*upper* traces) of TTX during ramp depolarizations from holding potentials of −59 and −78 mV. TTX increased the net outward holding current as the holding current was decreased from −78 mV. *B*, difference currents during ramp depolarizations from holding potentials of −49, −59, −68, and −78 mV (*inset*) are presented such that the difference currents are aligned for any given ramp potential. The difference current during the ramp depolarization increases as the holding potential is hyperpolarized over the range where sodium channel inactivation occurs. [CsCl] = 10 mM, [TTX] = 2×10^{-5} M.

of experimental conditions have suggested the existence of slow or incomplete inactivation of sodium channels which proceed over tens to hundreds of milliseconds (Adelman and Palti, 1969; Peganov et al., 1973; Rudy, 1975; Meves, 1978; Shoukimas and French, 1980). The effects of TTX on membrane currents of canine cardiac Purkinje fibers during square pulse and ramp depolarizations suggest that an analogous slow component of sodium channel inactivation is present under physiological conditions in cardiac fibers. We cannot rule out the possibility of two populations of cardiac sodium channels with different inactivation kinetics.

Some voltage clamp studies of cardiac preparations fail to find a second component of sodium channel inactivation (Ebihara and Johnson, 1980; Colatsky, 1980). Assuming a 600 V/s maximum rate of rise for the action potential upstroke and a membrane capacitance of 1 μF/cm^2 suggests a peak inward sodium current of 0.6 mA/cm^2. The maximal time-dependent difference current (arbitrarily measured by comparing the difference currents at 500 ms and 10 s) is ~100 nA in our preparations (2 mm length by 0.3 mm diam, and a membrane-surface-to-apparent-preparation-surface ratio of 11.7 [Cohen et al., 1983]). This leads to an estimated time-dependent difference current of ~0.6×10^{-3} mA/cm^2. Assuming a homogeneous population of sodium channels, we estimate that the time-dependent sodium window current is 1,000-fold less than the fast inward sodium current. Even if only 10% of the preparation membrane area contributes to the rapid upstroke (Schoenberg et al., 1975), the peak window current density would be 100-fold less than peak inward sodium current density. Given the relative sizes of the two currents and the prolonged clamp durations required, it is not surprising that direct measurements of peak inward sodium current fail to reveal this small, time-dependent component.

Two components of inactivation are required to fit the relaxation of sodium current of voltage clamped single rat heart muscle cells (Brown et al., 1981). However, even this slow component of inactivation (τ_{h2} < 10 ms) is probably too fast to account for our findings. Studies with sheep Purkinje fibers (Saikawa and Carmeliet, 1982) and isolated rat myocardial cells (Brown et al., 1981; Zilberter et al., 1982) report evidence for both fast and slow reactivation of cardiac sodium channels, which indirectly suggests the possibility of a slow inactivation process.

Finally, it is worth considering the functional role of this time-dependent current in the control of the action poten-

tial duration. Slow inactivation of cardiac sodium channels may be important in sustaining the action potential plateau, as well as modulating the action potential duration following changes in the stimulation rate. Slow inactivation also may play a role in use-dependent block of cardiac sodium channels by local anesthetics (Johnson and McKinnon, 1957; Hondeghem and Katzung, 1977).

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