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Sodium Currents in Segments of Human Heart Cells

Abstract. *Isolated human heart cells were partially drawn into the lumen of a plastic tube and cleaved at the partitioning tube wall by intraluminal suction pulses. The extraluminal segment (10 to 20 percent of the cell length) was suitable for intracellular perfusion and voltage clamp. The time and voltage dependence of the sodium current, and the responses to changes in driving force and channel blockers, illustrate the potential of these preparations as models for the study of membrane channels.*

The complex structure and large membrane area of multicellular cardiac preparations preclude or greatly complicate the measurement of sodium current (I_{Na}) in the heart (*1*). A recent solution has been to apply voltage clamp techniques to single cells isolated from rat cardiac tissue (*2*, *3*). By employing a modification of a cell dialysis–voltage clamp technique (*3*, *4*), we measured I_{Na} in single myocytes isolated from human heart tissue.

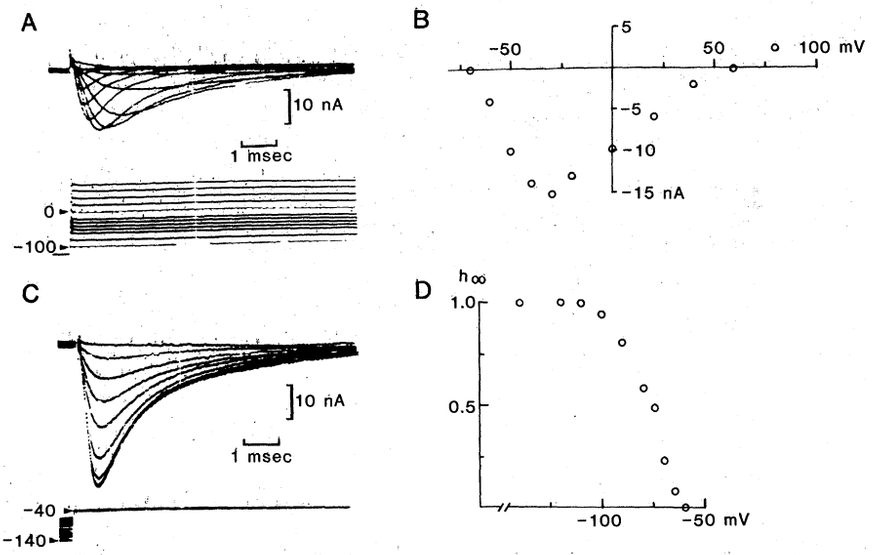

Fig. 1. Sodium current in human atrial cell segments. Pulsing rate was 0.2 Hz. (A) Currents accompanying 10-msec-long depolarizations from −120 mV. (B) Current-voltage relation of peak I_{Na} from the records shown in (A). Peak I_{Na} was measured with reference to zero current. (C) I_{Na} in a different cell. Test pulses to −40 mV were preceded by 100-msec pulses from −120 mV to potentials between −140 and −60 mV. (D) Steady-state inactivation of I_{Na} measured from the records in (C).

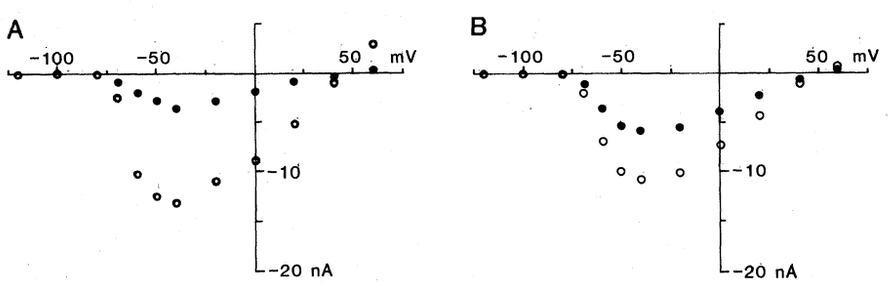

Fig. 2. Depression of I_{Na} after 5-minute exposure to sodium channel blockers. Experimental protocol was similar to that in Fig. 1, A and B. (○) Controls; (●) 5 μ*M* TTX in (A) and 250 μ*M* lidocaine in (B).

Cells were isolated from nondiseased atrial specimens obtained during corrective cardiac surgery (*5*). They were collected during the first 30 to 45 minutes of tissue digestion with collagenase V (300 U/ml; Sigma) and protease VII (1.0 U/ml; Sigma) in calcium-free solution. The calcium-free solution contained (millimoles per liter): 150.0 NaCl, 2.5 CsCl, 2.5 Cs-Hepes, 2.5 Hepes–free acid, 1.0 $MgCl_2$, and 10.0 glucose (pO_2 100 to 150 mmHg, *p*H 7.3). The isolated cardiocytes are about 100 μm long and 10 μm wide, have a morphology similar to that of cells dispersed from laboratory animal hearts (*6*), and display simultaneous action potentials and contractions after electrical stimulation (*5*).

The apparatus for intracellular perfusion and voltage clamp was similar to that described by Kostyuk *et al.* (*4*). A pore 2 to 4 μm narrower than the width of the cell to be studied was bored through the 4- to 6-μm wall of the V-shaped plastic tube. Pore resistance was 100 to 200 kilohms and accounted for most of the system series resistance. Series resistance compensation was achieved by conventional means (*4*).

The cell and tube were viewed through the glass bottom of a 1-ml bath with a modified, inverted microscope at ×100 to ×800 magnification. After the pore was positioned near one end of the cell, a small negative hydrostatic pressure of 5 to 10 mmHg was applied to the interior of the tube to attract the cell to the pore. An increase in negative pressure resulted in the aspiration of 80 to 90 percent of the cell into the lumen of the tube, and a series of suction pulses (about 400 mmHg, 1 second) sheared off the intraluminal cell segment.

The intracellular perfusate flowing in the tube contained (millimoles per liter): 150.0 CsF, 10.0 NaF, 2.5 Cs-Hepes, and 2.5 Hepes–free acid (*7*). The bath was perfused with calcium-free solution for 5 minutes before and during and for 5 minutes after cell aspiration into the tube; this prevented suction-induced contracture. Recovery of the test segment was then facilitated by addition of 1.0 m*M* $CaCl_2$ to the external solution and by application of hyperpolarizing currents (1 to 30 nA). After 1 to 3 minutes, a current of less than 1 nA was sufficient to maintain a stable membrane potential (V_m) of −120 mV.

Experiments were performed at 20° to 22°C and the preparation was usually stable for 30 to 90 minutes. To suppress calcium current, $MnCl_2$ (3 m*M*) was added to the external solution. Electrical signals were filtered at 10 kHz, displayed on a storage oscilloscope (Tektronix

5113), and recorded on 35-mm film.

Membrane currents recorded from human atrial cell segments are shown in Fig. 1. With the holding potential (V_H) set at −120 mV, 10-msec-long voltage pulses triggered inward I_{Na} at V_m positive to −70 mV; the current reversed direction at +60 mV (Fig. 1, A and B). In additional experiments, changes in external or internal sodium concentration ($[Na]_o$ or $[Na]_i$) altered I_{Na} and its reversal potential in a manner similar to that observed in studies on nerve axons (*8*).

The steady-state inactivation variable (h_∞) was estimated by measuring the effect of 100-msec-long prepulses (−140 to −60 mV) on test I_{Na} at −40 mV (Fig. 1, C and D). Steady-state inactivation was negligible at potentials negative to −100 mV, reached one-half at about −75 mV, and was complete at about −60 mV.

The final series of experiments showed that I_{Na} in human cardiocytes is sensitive to the external application of sodium channel blocking agents. After 5 μ*M* tetrodotoxin (TTX; Sigma) for 5 minutes, peak I_{Na} was reduced by about 75 percent (Fig. 2A). Similar exposure to 250 μ*M* lidocaine (Astra) blocked about 50 percent of the current (Fig. 2B) (*9*).

It is apparent that the maximum peak I_{Na} in human cardiocytes was much smaller than that recorded in other cardiac preparations (*2, 3, 10–13*). The reason lies in the small area of the test membrane, since our estimates of current densities, 1 to 5 mA/cm^2, agree with those for rat heart cells (*12*). The threshold potential (−60 to −70 mV), V_m of maximum peak I_{Na} (~ −30 mV), and reversal potential (~ +60 mV at $[Na]_o/[Na]_i = 15$) also coincide, but V_m for $h_\infty = 0.5$ (mean ± standard deviation, −77 ± 6 mV; $N = 5$) is about 10 mV more negative than estimates for rat cardiocytes at the same temperature (*12*). The h_∞ discrepancy with the rat cells may be related to the rather short (40 msec) prepulses used in that study, since our h_∞-V_m curves almost coincide with those determined in rabbit Purkinje fibers with prepulses of 200 to 500 msec (*10*). Finally, the degree of I_{Na} block with TTX or lidocaine treatment is in line with that found by others using similar pulsing protocols (*12, 13*).

We conclude that the internally perfused, heart cell segment is a suitable experimental model for the study of membrane ionic channels in the heart.

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Natural Distribution of the *Ixodes dammini* Spirochete

Abstract. *Spirochetes believed to be the cause of Lyme disease were isolated from white-footed mice and white-tailed deer, the preferred natural hosts of* Ixodes dammini, *the tick vector. Evidence suggests that deer act as a reservoir of the disease and provide an overwintering mechanism for both spirochetes and adult ticks. Some tick larvae may acquire the spirochete by transovarial passage and the nymphal stage may transmit the disease to humans.*

Lyme disease, an epidemic inflammatory disorder with a pathognomonic skin lesion that is often followed by joint, neurological, and cardiac manifestations, was first recognized in 1975 in Lyme, Connecticut (*1*). Epidemiologic evidence for the Northeast suggests that nymphal *Ixodes dammini* ticks transmit the causative agent of Lyme disease to humans (*2*). In 1981 spirochetes thought to be the agent were isolated from adult *I. dammini* ticks, which infest a wide variety of avian and mammalian hosts (*3, 4*). Immature ticks or subadults feed on rodents, primarily white-footed mice (*Peromyscus leucopus*), and also on white-tailed deer (*Odocoileus virginianus*) (*5*). Adults feed on white-tailed deer in the spring and fall (*4*). All stages of *I. dammini* have been reported to feed on humans (*6*).

We isolated and identified spirochetes believed to be the agent of Lyme disease from the blood of feral *P. leucopus* and *O. virginianus* and from subadult *I. dammini* ticks removed from these mammals. Beginning in mid-June 1982, white-footed mice were collected weekly from the same endemic focus on Shelter Island, New York, that yielded the spirochete-infected adult *I. dammini* in 1981 (*3*). Of 77 *P. leucopus* trapped during an 8-week period, five had spirochetemia (Table 1). Dark-field examination of blood obtained aseptically by cardiac puncture failed to disclose spirochetes; it was only by culturing the whole blood samples in BSK medium (*7*) that spirochetes could be detected. The spirochetes were detected 10 to 31 days (mean, 19 days) after the blood (0.1 ml) was added to the medium (7 ml) (Table 2). It is not known whether the longer time required for growth in certain cultures was due to the presence of only small numbers of spirochetes at sampling time or to the presence of inhibitory substances in the rodents' blood. The rodent spirochetes have been routinely subcultured (*8*), and their identity as the previously reported *I. dammini* spirochete (*3*) was confirmed by direct immunofluorescence (*9*) and monoclonal antibody tests (*10*). The low frequency of blood spirochetes in the mice could indicate that *P. leucopus* is an inefficient reservoir or that the spirochetemia is transient and that timing factors are crucial for recovering the organisms from blood.

Of 306 *I. dammini* subadults, 113 (101 larvae and 12 nymphs) were infected with spirochetes (Table 1). The infected ticks were removed from 33 (43 percent) of the tick-infested *P. leucopus*. Since 23 of the infected larvae were unengorged, that is, without blood in their midgut, the larvae may have acquired the spirochete through transovarial passage. The occurrence of transovarial transmission of the