Reduction of the Transient Outward Potassium Current in Canine X-Linked Muscular Dystrophy

Linda M. Pacioretty, PhD; Barry J. Cooper, PhD; Robert F. Gilmour, Jr, PhD

Background The xmd dog develops a cardiomyopathy similar to that seen in Duchenne muscular dystrophy patients. In both the canine and human diseases, ECG abnormalities may precede the development of overt cardiac pathological lesions. The purpose of this study was to determine whether specific cellular electrical abnormalities occur in dystrophic ventricular tissue.

Methods and Results Action potentials were recorded in epichardial tissue strips obtained from normal and xmd dogs. Phase 1 amplitude was increased from 86.8±2.7 mV in normal dogs to 94.3±1.8 mV in xmd dogs (mean±SEM; P<.05). The 4-aminopyridine-sensitive transient outward potassium current (Iₒ), as recorded in isolated epichardial myocytes using the whole-cell patch-clamp technique, was reduced in xmd dogs compared with age-matched normal dogs. Cell capacitance also was reduced significantly in xmd compared with normal cells, as was the current density (3.6±0.3 versus 5.4±0.8 pA/pF, respectively). No differences were observed in the time constants of current decay or in the kinetics of recovery from inactivation between groups. The slope factor (k) of steady-state inactivation was significantly greater in xmd compared with normal cells (7.2±0.9 versus 5.4±0.5, respectively), whereas the V1/2 of inactivation did not differ (~38.2±2.4 versus ~36.8±1.6 mV, respectively).

Conclusions These data indicate that the magnitude of Iₒ is reduced in dystrophic epichardial myocytes, resulting in an increase in phase 1 amplitude. The reduction of Iₒ may alter the balance of inward and outward currents in dystrophic myocardium and thereby contribute to the development of cardiac pathology. (Circulation. 1994;90:1350-1356.)

Key Words • electrophysiology • ions • cardiovascular diseases • cardiomyopathy

The xmd dog is an animal model for Duchenne muscular dystrophy (DMD) in humans, an X-linked recessive disease affecting 1 in 3000 to 4000 births.4 The mutation responsible for this disease in both humans and dogs occurs in the gene encoding the 427-kD protein dystrophin.2-5-11 Dystrophin is a cytoskeletal protein normally found in smooth, skeletal, and cardiac muscle and in neural tissue.6,9-17 Its absence is associated with progressive muscle necrosis and wasting and eventual death from respiratory or cardiac failure.1,18-24 Dystrophin is localized to the cytoplasmic face of the plasma membrane and forms a complex with four glycoproteins and one 59-kD protein.10,14,25-29 Although the function of dystrophin is unclear, it has been hypothesized that it provides a link between the intracellular cytoskeleton and extracellular matrix and that it contributes to stabilization of the complex of associated proteins.27,30 Recent studies have shown that the absence of dystrophin results in a deficiency of dystrophin-associated proteins.26,27,30 Whether the absence of dystrophin itself and/or the loss the associated proteins initiates the disease process remains to be determined.

The cardiac pathological features in X-linked muscular dystrophy have become an important consideration in the life expectancy of DMD patients. The absence of dystrophin in human and canine hearts results in progressive myocardial degeneration, fibrosis, and necrosis, evolving in a characteristic pattern throughout the subepicardium and the posterobasal left ventricular free wall, with relative sparing of the subendocardium.31 Whether the rhythm and conduction disturbances present in dystrophic humans and dogs31 are the direct result of the absence of dystrophin or merely a consequence of the disease-related fibrosis and necrosis is unknown. The observation that electrical abnormalities are present early in the disease process22,23 suggests that a primary electrophysiological defect may predate the development of necrosis. One candidate for such a defect is an alteration in the transient outward potassium current (Iₒ), given that this current is enriched in the subepicardium and is virtually absent in the endocardium.34 To determine whether Iₒ is altered in X-linked muscular dystrophy, we measured Iₒ in epichardial myocytes isolated from normal and from xmd dogs of different ages and correlated changes in Iₒ with alterations of phase 1 repolarization.

Methods

Action Potential Measurements

Golden retriever crosses of either sex obtained from a single colony of reared dogs and ranging in age from 16 weeks to adult were anesthetized with Fatal-Plus (390 mg/mL pentobarbital sodium; Vortech Pharmaceuticals; 86 mg/kg IV). Hearts were removed rapidly via a left thoracotomy and were placed in cold oxygenated (95% O₂,5% CO₂) Tyrode's solution containing (in mmol/L): MgCl₂ 0.7, NaH₂PO₄ 0.9, CaCl₂ 2.0, NaCl 124, NaHCO₃ 24, KCl 4, glucose 5.5, pH 7.4. A section of the right ventricular free wall approximately 5×5 mm was removed and placed in a Plexiglas superfusion chamber. The preparations were superfused at a rate of 15 mL/min with continuously oxygenated Tyrode's solution. The temper-
TABLE 1. Action Potential Parameters

<table>
<thead>
<tr>
<th>Dog</th>
<th>n</th>
<th>Drug</th>
<th>dV/dt_max, V/s</th>
<th>APA, mV</th>
<th>PhIA, mV</th>
<th>APD50, ms</th>
<th>APD90, ms</th>
<th>RMP, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>Prop</td>
<td>199.6±12.4</td>
<td>103.1±1.3</td>
<td>86.8±2.7</td>
<td>130.0±2.3</td>
<td>160.0±3.1</td>
<td>−87.6±1.3</td>
</tr>
<tr>
<td>8</td>
<td>4-AP+Prop</td>
<td>170.2±11.4</td>
<td>103.0±1.3</td>
<td>100.0±1.6†</td>
<td>136.2±3.5</td>
<td>171.8±3.9†</td>
<td>−87.8±1.4</td>
<td></td>
</tr>
<tr>
<td>xmd</td>
<td>7</td>
<td>Prop</td>
<td>190.6±24.9</td>
<td>106.7±2.2</td>
<td>94.3±1.8*</td>
<td>130.0±6.4</td>
<td>168.1±4.2</td>
<td>−88.0±1.0</td>
</tr>
<tr>
<td>7</td>
<td>4-AP+Prop</td>
<td>176.1±25.0</td>
<td>106.7±1.1</td>
<td>102.4±1.1†</td>
<td>143.6±4.9†</td>
<td>186.4±3.5†</td>
<td>−87.7±0.9</td>
<td></td>
</tr>
</tbody>
</table>

APA indicates action potential amplitude; PhIA, phase 1 amplitude; APD50 and APD90, action potential duration at 50% and 90% repolarization, respectively; RMP, resting membrane potential; Prop, propanolol; and 4-AP, 4-aminopyridine.

*P<.05 vs Normal; †P<.05 vs Prop.

The action potential parameters measured were maximum upstroke velocity (dV/dt_max), action potential amplitude (APA), phase 1 amplitude (PhIA, the difference between the resting membrane potential and the minimum voltage during Ph1), action potential duration at 50% repolarization (APD50), action potential duration at 90% repolarization (APD90), and resting membrane potential (RMP). PhIA was measured because rapid repolarization during this portion of the action potential is believed to reflect activation of the transient outward current. The steady-state relation between PhIA or APD and pacing cycle length was examined for cycle lengths ranging from 200 to 2000 milliseconds. The restitution of APA, PhIA, APD50, and APD90 were examined by delivering a premature stimulus (S2) at S1-S2 intervals ranging from 170 to 1800 milliseconds after stimulation at an S1-S2 of 500 milliseconds. Measurements were obtained during superfusion with normal Tyrode’s solution or after 15 minutes of superfusion with normal Tyrode’s solution containing 2 mmol/L 4-aminopyridine (4-AP; Sigma Chemical Co), 30 to 60 minutes of superfusion with normal Tyrode’s solution containing 1 mmol/L propanolol, or 15 minutes of superfusion with 2 mmol/L 4-AP and 1 mmol/L propanolol. A stock solution of 50 mmol/L 4-AP dissolved in 0.5 mmol/L HEPES was diluted in the Tyrode’s solution to obtain the final concentration. This concentration of the vehicle previously has been shown to have significant electrophysiological effects. Because 4-AP may cause the release of endogenous catecholamines, which may, in turn, affect action potential characteristics via stimulation of β-adrenergic receptors, propanolol was included in the Tyrode’s solution. Propanolol had no significant effect on any of the action potential characteristics in either group (data not shown).

Cell Preparation

In all animals, the circumflex coronary artery or a branch of the left anterior descending coronary artery was cannulated and a portion of the left ventricle excised. The cannulated segment was placed in a tissue bath and perfused at a constant flow rate of 18 mL/min with oxygenated solutions maintained at 37°C. The heart tissue initially was perfused with Tyrode’s solution. After 10 to 15 minutes, the perfusion was switched to a Ca²⁺-free solution containing (in mmol/L): NaCl 118, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.2, glutamine 0.68, glucose 11, NaHCO₃ 25, pyruvate 5, mannitol 2, taurine 10, pH 7.3. At approximately 3 to 5 minutes, collagenase (type II, Worthington Biochemical Corp; 0.4 mg/mL) and bovine serum albumin (BSA, 0.5 mg/mL; Sigma Chemical Co) were added and the perfusion switched to the recirculating mode for 10 to 15 minutes, depending on the age of the dog. Hearts from younger animals were perfused for the shorter times. Digested tissue was sliced away from the subepicardial area and placed in 10 mL of enzyme solution and swirled. The supernatant was collected, and 10 mL of fresh calcium-free solution with 0.4 mg/mL collagenase and 0.5 mg/mL BSA was added to the slurry and gently bubbled in a water bath maintained at 37°C. Supernatant was collected for six subsequent washes at varying time intervals, depending on subjective evaluation of digestion (cloudiness of supernatant). After final pellet formation, the supernatant was removed and the pellet washed in 10 mL of incubation buffer containing (in mmol/L): NaCl 118, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.2, glutamine 0.68, glucose 11, NaHCO₃ 20, HEPES 5, pyruvate 5, taurine 10, CaCl₂ 0.5, and 2% BSA. After 30 minutes of settling, the supernatant was again removed, and the pellet was washed a second time with incubation buffer, now containing 1 mmol/L CaCl₂, and allowed to equilibrate at room temperature for 30 minutes. The cells selected for study from either group were rod shaped and quiescent and had clearly demarcated myofibrils.

![Image of Figure 1](https://example.com/image1.png)

**Fig 1.** Representative action potential (upper trace) and dV/dt (lower trace) recordings obtained from normal epicardium before (A) and after (C) the addition of 2 mmol/L 4-aminopyridine (4-AP) and from xmd epicardium before (B) and after (D) the addition of 2 mmol/L 4-AP. The pacing cycle length was 500 milliseconds. Vertical and horizontal calibrations for action potentials are 40 mV and 100 milliseconds, respectively. Vertical and horizontal calibrations for dV/dt recordings are 200 μV and 10 milliseconds, respectively.
were for discussion.

**Voltage-Clamp Technique**

Current was measured in the whole-cell configuration as previously described with an Axopatch-ID (Axon Instruments) interfaced with a personal computer (Dell System 320LX). Data acquisition and analysis was performed with a commercial program (pClAMP, version 5.5, Axon Instruments). Seals were made in Tyrode’s solution, and all experiments were performed at 22°C to 25°C. Pipettes were pulled from Gold Seal Accu-fill 90 Micropets (Clay Adams) and had resistances of 2 to 5 MΩ when filled with internal solution. Series resistances from the pipette tip and cell capacitance were partially compensated, as was the liquid junctional potential between the pipette and the bath solutions (typically 5 to 10 mV). Alterations in the reference potential were minimized by connecting the bath to the reference potential well (containing internal solution) with an agar bridge. Current records were filtered at 2 kHz and sampled at a frequency of 10 kHz.

I\textsubscript{o} was measured in a Na\textsuperscript{+}-free bath solution containing (in mmol/L): N-methylglucamine 125, KCl 4, HEPES 10, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 0.1, glucose 5, Cd\textsuperscript{2+} 0.2, tetrodotoxin 0.1 (TTX; Sigma Chemical Co.), pH 7.3. The pipette solution contained (in mmol/L): potassium aspartate 125, KCl 20, EGTA 10, ATP (magnesium salt) 5, MgCl\textsubscript{2} 1, HEPES 5, pH 7.3. Activation of I\textsubscript{o} was recorded using test pulses to −70 to +60 mV from a holding potential of −80 mV. The duration of the depolarizing pulses was 300 milliseconds, and the amplitude of I\textsubscript{o} was measured as the difference between peak outward current and minimum current during the depolarizing pulse after the peak. By recordings at 22°C to 25°C, the peak outward current was easily distinguished from the capacitative current, and there was little contribution of I\textsubscript{o} to the steady-state current. To identify the 4-AP–sensitive current, 2 to 20 mmol/L 4-AP was added in one to four experiments from each group.

The decay of I\textsubscript{o} was analyzed by a single exponential fit of the current recorded at +40 mV. The voltage dependence of steady-state inactivation was determined by a double-pulse protocol, whereby a 500-millisecond conditioning pulse, ranging from −80 to +40 mV, was followed by a 300-millisecond test pulse to +40 mV. The results were fitted to a Boltzmann function,

\[ I/I_{\text{max}} = 1/(1 + \exp[(V_{1/2} - V_m)/k]) \]

by a least-squares method of nonlinear regression analysis. V\textsubscript{1/2} is the membrane potential at which half-inactivation occurs, and k is the slope factor at V\textsubscript{m} = V\textsubscript{1/2}. Recovery from inactivation was examined with a double-pulse protocol, in which the time interval between two 300-millisecond pulses to +40 mV from a holding potential of −80 mV was varied between 5 and 800 milliseconds. Cell capacitance was measured by integration of the area beneath the capacitative transient elicited by 10 mV depolarizing or hyperpolarizing steps and division of that area by the change in voltage. All current measurements were obtained from myocytes isolated from the subepicardium, hereafter referred to as epicardial myocytes.

The hearts from adult xmd dogs show marked degeneration, fibrosis, and calcification. To separate electrical abnormalities that resulted primarily from the absence of dystrophin per se from those that might have resulted from necrosis and progressive fibrosis, membrane currents and action potentials were recorded in young dogs ranging in age from 16 to 35 weeks that were relatively free of overt disease and clinical signs of heart failure and in adult dogs ranging in age from 45 weeks to >1 year. We have shown previously that I\textsubscript{o} increases with age from 10 weeks to adult and that current density increases from 10 to 20 weeks and remains constant thereafter. In addition, the time constant of current decay increases with age, whereas the kinetics of steady-state inactivation and recovery from inactivation do not change with age. Accordingly, the measurements of current magnitude, current decay, cell capacitance, and cell size were compared at both ages for normal and xmd dogs to eliminate age-related differences in these parameters. The results for the age-independent parameters were combined for all age groups, and comparisons were made between normal and xmd dogs. Because of the X-linked inheritance of the disease, truly unaffected dogs are rarely produced by this colony. Consequently, no normal adult dogs (45 weeks to >1 year of age) were available for this study.

**Statistical Analysis**

Data are reported as mean ± SEM except as noted. Statistically significant differences between groups were evaluated by
ANNOVA (STATVIEW, Abacus Concepts) followed by Scheffé’s F test. *P* < .05 was considered statistically significant.

**Results**

**Action Potential Characteristics**

The steady-state action potential characteristics of normal and dystrophic cells during superfusion with propranolol (1 μmol/L) are summarized in Table 1. The pacing cycle length was 500 milliseconds. Action potentials recorded in normal and xmd dogs were similar with respect to rate of rise (dV/dt max), APA, RMP, and APD 90 and APD 50 . Ph1A was significantly greater in xmd dogs than in normal dogs (Fig 1).

To demonstrate the sensitivity of Ph1A to blockade of I Ca, the effects of 4-AP (2 mmol/L) were determined. After the addition of 4-AP, Ph1A increased to similar values for both groups. 4-AP also increased APD 90 and APD 50 in xmd tissue and APD 90 in normal tissue. APA, RMP, and dV/dt max were not altered significantly by 4-AP, although dV/dt max tended to be decreased.

The mean data for restitution of Ph1A in control and xmd epicardium before and after exposure to 4-AP (2 mmol/L) are given in Fig 2. Ph1A was greater in xmd than in control tissue at coupling intervals greater than 400 milliseconds in the absence of 4-AP and was similar at all coupling intervals in both groups after the addition of 4-AP. Restitution of Ph1A followed a biexponential time course in five of seven experiments in xmd tissue and in eight of nine experiments in control tissue. For experiments in which a biexponential time course of restitution occurred, no differences were apparent between normal and xmd dogs in the time constants for either the rapid phase (τ 1 ) or the slower phase (τ 2 ) of recovery (τ 1 = 69.8±9.8 and 93.8±11.3 milliseconds, respectively; τ 2 = 339.5±78.2 and 439.6±86.8 milliseconds, respectively). For those experiments in which a monoexponential time course of Ph1A restitution occurred, the time constants were similar to the fast time constants observed during biexponential restitution.

**Transient Outward Current**

To determine whether the increase of Ph1A in dystrophic epicardium reflected a reduction in I Ca, the 4-AP-sensitive component of I Ca was measured in normal and dystrophic myocytes. This current initially was identified by subtracting the currents obtained before and after exposure to 4-AP (Fig 3). Because the magnitude of the 4-AP-sensitive current was similar to estimates of I Ca obtained by measuring the difference between the peak and steady-state current during the clamp step, as described above, the latter measurements were used for comparing I Ca between groups.

The activation threshold for I Ca was similar in both dystrophic and normal myocytes, at −25 to −30 mV, but the magnitude of I Ca in dystrophic myocytes was reduced compared with normal myocytes throughout the cur-

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**Fig 4.** I Ca recorded from a normal epicardial myocyte (left) and an xmd epicardial myocyte (right) during 300-millisecond depolarizing steps from −20 to +60 mV, from a holding potential of −80 mV. Current traces are not leak subtracted; capacitative current was blanked. Arrows indicate zero current.

**Fig 5.** A, Mean current-voltage relations for I Ca recorded from normal (solid circles) (n=22) and xmd (open circles) (n=23) myocytes at 16 to 35 weeks of age. B, Mean current-voltage relations for normalized I Ca for normal and xmd myocytes.
current-voltage relation for dogs ranging in age from 16 to 35 weeks (Figs 4 and 5A). Cell capacitance was significantly reduced in xmd myocytes compared with age-matched normal myocytes (60.9±3.7 vs 82.5±4.8 pF, respectively; Fig 6). To ensure that the reduced magnitude of \( I_{o} \) in dystrophic myocytes did not result solely from the smaller size of these cells, current measurements were normalized to cell capacitance. After normalization, \( I_{o} \) remained significantly lower in dystrophic myocytes compared with normal myocytes (Figs 5B and 6).

A significant age-related increase in the magnitude of \( I_{o} \) occurred in xmd myocytes; peak \( I_{o} \) at +40 mV was 200.5±20.0 pA at 16 to 35 weeks versus 418.1±84.7 pA at >45 weeks (Fig 6). The age-related increase in \( I_{o} \) in dystrophic myocytes was accompanied by an increase in cell size, as estimated by whole-cell capacitance (60.9±3.7 pF at 16 to 35 weeks versus 109.9±11.2 pF at >45 weeks; Fig 6). The parallel age-related increases of \( I_{o} \) and cell capacitance were reflected in no change of \( I_{o} \) density (Fig 6).

The kinetic properties of \( I_{o} \) also were compared between xmd and normal myocytes. The time constants of current decay (\( \tau \)) were examined at +40 mV by a single exponential fit of the current from its maximum value to 135 milliseconds after peak current. The \( \tau \) did not differ between normal and xmd myocytes, averaging 26.1±1.6 and 26.4±1.5 milliseconds, respectively.

The voltage dependence of steady-state inactivation was described by a Boltzmann function. The inactivation of \( I_{o} \) normalized to maximum current followed a sigmoidal shape in both normal and xmd myocytes, with inactivation complete at 0 mV and almost completely removed at membrane potentials more negative than -65 mV and -75 mV for normal and xmd cells, respectively (Fig 7). \( V_{1/2} \) did not differ between dystrophic and normal myocytes, whereas \( k \) was slightly but statistically significantly greater for the dystrophic myocytes (Table 2).

Recovery from inactivation for \( I_{o} \) was best fit with a biexponential function, as illustrated in Fig 8, in which the fraction of current that was activated during the second pulse of a double-pulse protocol is shown as a function of the interpulse interval. The \( \tau \) of both the rapid and slow phases of recovery did not differ between normal and xmd myocytes (Table 2) and were similar to the slow and fast time constants of restitution of Ph1A.

### Discussion

**Cellular Electrophysiological Alterations in X-Linked Muscular Dystrophy**

Action potentials recorded from dystrophic epicardium differed significantly from normal epicardium only

<table>
<thead>
<tr>
<th>Dog</th>
<th>( k )</th>
<th>( V_{1/2}, \text{mV} )</th>
<th>( \tau_{1}, \text{ms} )</th>
<th>( \tau_{2}, \text{ms} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.4±0.5</td>
<td>-36.8±1.6</td>
<td>54.2±10.7</td>
<td>348.1±23.3</td>
</tr>
<tr>
<td>xmd</td>
<td>7.2±0.9*</td>
<td>-38.2±2.4</td>
<td>77.3±9.7</td>
<td>383.7±24.1</td>
</tr>
</tbody>
</table>

*\( V_{1/2} \) indicates membrane potential at which half-inactivation occurs; \( \tau_{1} \), time constant of rapid phase of recovery; and \( \tau_{2} \), time constant of slower phase of recovery.

*\( P<.05 \) vs Normal.
Fig 8. Example of the recovery from inactivation of $I_{\text{Na}}$ in a normal (solid circles) and an xmd (open circles) epicardial myocyte. Curves are fitted biexponential functions. Insets show voltage-clamp protocol and current traces at interpulse intervals of 150 to 800 milliseconds.

with respect to phase 1 repolarization, suggesting that X-linked dystrophy initially is associated with a specific cellular electrophysiological defect as opposed to a more generalized loss of membrane integrity. The increase in PhlA was accompanied by a corresponding reduction of $I_{\text{Na}}$. Although a reduction of $I_{\text{Na}}$ is the most straightforward explanation for the increase in PhlA in xmd cells, it is possible that alterations of other ionic currents may have contributed to this effect. Moreover, offsetting alterations of inward and outward plateau currents may have occurred without producing significant changes in action potential morphology. Nevertheless, our results indicate that dystrophin evidently is not essential for the function of $I_{\text{Na}}$ (i.e., dystrophin was completely absent in the dystrophic cells, whereas $I_{\text{Na}}$ was only attenuated), the lack of dystrophin is associated with a specific electrophysiological defect in cardiac cells.

The lower magnitude of $I_{\text{Na}}$ in dystrophic myocytes resulted in part from the smaller size of these cells compared with normal myocytes. However, the size differential between normal and dystrophic cells was not solely responsible for differences in the magnitude of $I_{\text{Na}}$ in that current density was significantly reduced in xmd versus normal myocytes. The kinetic properties of $I_{\text{Na}}$ in xmd and normal myocytes were similar, with the exception of the slope factor of steady-state inactivation, which was slightly greater in dystrophic than in normal myocytes. However, the increase in steady-state inactivation of $I_{\text{Na}}$ at voltages in the range of the RMP was too small to account for the reduction of $I_{\text{Na}}$. Moreover, the $V_{1/2}$ did not differ between dystrophic and normal cells. Consequently, it is unlikely that the reduction in $I_{\text{Na}}$ seen in dystrophic cells was a consequence of altered macroscopic current kinetics.

Possible Contribution of Changes in $I_{\text{Na}}$ to Electrical and Contractile Abnormalities in the Dystrophic Heart

The ECGs recorded from DMD patients and xmd dogs are distinctive from other cardiac diseases in the commonly observed anterior shift of the QRS complex and abnormally deep but narrow Q waves. The relation between these electrical abnormalities and X-linked cardiomyopathy is believed to result from loss of electromotive forces in the diseases posteroventral area. Although $I_{\text{Na}}$ has been implicated in the production of certain components of the ECG, such as the J wave, it seems unlikely that a reduction of $I_{\text{Na}}$ would alter electrical forces to the extent that the Q wave was affected. Moreover, the increase in PhlA in dystrophic epicardium was not accompanied by other phenomena (e.g., very slow conduction, afterdepolarizations, or abnormal automaticity) that might account for the development of ventricular arrhythmias in dystrophic hearts. Thus, it appears unlikely that the loss of $I_{\text{Na}}$ contributes directly to the development of electrical abnormalities in X-linked muscular dystrophy. However, as discussed below, altered $I_{\text{Na}}$ may contribute indirectly to the development of such abnormalities by modulating the process of cellular necrosis.

A reduction of $I_{\text{Na}}$ has been associated with other cardiac diseases, such as terminal heart failure in humans and myocardial infarction and Chagas’ disease in dogs. Whether alteration of $I_{\text{Na}}$ contributes importantly to the development of electrical and contractile abnormalities in these disease states is or is simply the earliest electrophysiological consequence of disturbances in the cellular metabolic environment is unclear. In support of the first possibility, the rather long-term courses of some of the cardiac diseases that have been associated with a reduction in $I_{\text{Na}}$ are suggestive of a persistent, subtle alteration in cell function. With respect to DMD in particular, elevations in intracellular calcium levels are believed to be important mediators of the progressive cellular degeneration, possibly via activation of proteases. The mechanism for the apparent intracellular calcium overload is unknown. Perhaps a reduction in $I_{\text{Na}}$ distorts the balance between outward plateau currents and inward calcium current, resulting in a beat-to-beat increase in calcium influx and ultimately in pathological consequences. In this regard, inhibition of $I_{\text{Na}}$ has been shown to result in secondary increases of calcium current, intracellular calcium transients, and contractile force development.

Acknowledgments

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