Abnormal Cardiac Na\(^+\) Channel Properties and QT Heart Rate Adaptation in Neonatal Ankyrin\(_B\) Knockout Mice

Vijay S. Chauhan, Shmuel Tuvia, Mona Buhusi, Vann Bennett, Augustus O. Grant

**Abstract**—The cytoskeleton of the cardiomyocyte has been shown to modulate ion channel function. Cytoskeletal disruption in vitro alters Na\(^+\) channel kinetics, producing a late Na\(^+\) current that can prolong repolarization. This study describes the properties of the cardiac Na\(^+\) channel and cardiac repolarization in neonatal mice lacking ankyrin\(_B\), a cytoskeletal “adapter” protein. Using whole-cell voltage clamp techniques, \(I_{\text{Na}}\) density was lower in ankyrin\(_B\)-deficient ventricular myocytes than in wild-type (WT) myocytes (\(-307\pm26\) versus \(-444\pm39\) pA/pF, \(P<0.01\)). Ankyrin\(_B\)-deficient myocytes exhibited a hyperpolarizing shift in activation and inactivation kinetics compared with WT. Slower recovery from inactivation contributed to the negative shift in steady-state inactivation in ankyrin\(_B\)-deficient. Single Na\(^+\) channel mean open time was longer in ankyrin\(_B\)-deficient versus WT at test potentials \(V_t\) of \(-40\) mV (\(1.0\pm0.1\) versus \(0.61\pm0.04\) ms, \(P<0.05\)) and \(-50\) mV (\(0.8\pm0.1\) versus \(0.39\pm0.05\) ms, \(P<0.05\)). Ankyrin\(_B\)-deficient exhibited late single-channel openings at \(V_t\) \(-40\) and \(-50\) mV, which were not seen in WT. Late \(I_{\text{Na}}\) contributed to longer action potential durations measured at 90% repolarization (APD\(_{90}\)) at 1 Hz stimulation in ankyrin\(_B\)-deficient compared with WT (354\pm26 versus 274\pm22 ms, \(P<0.05\)). From ECG recordings of neonatal mice, heart rates were slower in ankyrin\(_B\)-deficient than in WT (380\pm14 versus 434\pm13 bpm, \(P<0.01\)). Although the QT interval was similar in ankyrin\(_B\)-deficient and WT at physiological heart rates, QT-interval prolongation in response to heart rate deceleration was greater in ankyrin\(_B\)-deficient. In conclusion, Na\(^+\) channels in ankyrin\(_B\)-deficient display reduced \(I_{\text{Na}}\) density and abnormal kinetics at the whole-cell and single-channel level that contribute to prolonged APD\(_{90}\) and abnormal QT-rate adaptation. ([Circ Res. 2000;86:441-447.])

**Key Words:** Na\(^+\) channel ■ repolarization ■ transgenic mice ■ cytoskeleton ■ long-QT syndrome

Ankyrins are a family of cytoskeletal “adapter” proteins that bind to integral membrane proteins, including ion channels such as the Cl/HCO\(_3\)-exchanger, Na\(^+\)/K\(^+\)-ATPase, Na\(^+\)/Ca\(^2+\) exchanger, ryanodine receptor, inositol trisphosphate receptor, and voltage-gated Na\(^+\) channel. Ankyrins play a key role in sustaining the structural integrity of the cell as well as localizing functionally distinct proteins to specific sites in the plasma membrane and intracellular membranes (for review, see Bennett and Gilligan). In the heart, ankyrin\(_B\) is present in (1) the sarcolemma in a costamere pattern, which represents sites of attachment of the sarcolemma to sarcomeres, and (2) the sarcoplasmic reticulum (SR).

Ankyrin\(_B\) is essential for correct intracellular assembly of Ca\(^2+\) homeostasis proteins to the SR of cardiomyocytes. We have previously described the phenotype of neonatal ankyrin\(_B\) knockout (\(-/-\)) mice that lack ankyrin\(_B\). Four-day-old ankyrin\(_B\)-deficient ventricular myocytes exhibit abnormal cytosolic Ca\(^2+\) waves due to abnormal localization of ryanodine receptors, inositol trisphosphate receptors, and SR-Ca\(^2+\) ATPase.

The role of ankyrin\(_B\) in sarcolemma ion channel function and regulation is unknown. The absence of ankyrin\(_B\) in the costamere of ankyrin\(_B\)-deficient cardiomyocytes likely destabilizes the sarcolemma and the underlying cytoskeleton. Disruption of the cardiomyocyte cytoskeleton can modify the kinetics of the Na\(^+\), Ca\(^2+\), and K\(_{\text{ATP}}\) channel. Similarly, ion channel kinetics may be altered in ankyrin\(_B\)-deficient cardiomyocytes. With respect to the Na\(^+\) channel, Undrovinas et al have demonstrated that breakup of the actin-based cardiomyocyte cytoskeleton with cytochalasin D produced late Na\(^+\) current at hyperpolarizing test potentials. Late Na\(^+\) current has been shown to prolong cardiac repolarization in long-QT syndrome (LQTS) patients who carry the inactivation-deficient Na\(^+\) channel mutations. Thus, it is a plausible hypothesis that ankyrin\(_B\)-deficient cardiomyocytes will have altered Na\(^+\) channel kinetics that may influence cardiac repolarization. In contrast, ankyrin\(_B\)-deficient cardiomyocytes that have reduced cardiac ankyrin\(_B\) levels when compared with wild-type (WT) may exhibit an intermediate Na\(^+\) channel phenotype.

The objectives of this study were to compare (1) the properties of the Na\(^+\) channel in ankyrin\(_B\)-deficient, ankyrin\(_B\)-deficient, and WT 1-day-old ventricular myocytes using whole-cell and single-channel voltage-clamp techniques and (2) the APD and QT interval of these mice using current clamp techniques and surface ECG recordings, respectively. It was
necessary to study neonates because of the limited life span of the knockout mice related in part to abnormal nervous system development.\(^2\) One-day-old cardiomyocytes were used because their SR-Ca\(^{2+}\) protein apparatus was still not fully developed (S. Tuvia, V. Bennett, unpublished observations, 1999), which would minimize differences in Ca\(^{2+}\) homeostasis between ankyrin\(_{b}(+/−)\) and WT myocytes.

**Materials and Methods**

**Knockout Mice**

WT, ankyrin\(_{b}(+/−)\), and ankyrin\(_{b}(−/−)\) mice were obtained from a breeding colony using animals generated by homologous recombination as previously described.\(^2\) For each mouse, genotyping was determined using Southern blot analysis.\(^8\) Animal protocols conformed to institutional guidelines.

**Single-Cell Studies**

Single ventricular myocytes were isolated from 1-day-old mice according to the neonatal cardiomyocyte isolation system (Worthington Biochemical Corp). Dispersed myocytes were cultured in medium containing 10% equine serum, 5% FBS, 50 \(\mu\)g/mL gentamicin, and DMEM/F-12 (1:1). All electrophysiological studies were performed 24 to 48 hr after isolation at room temperature (22\(^\circ\)C to 25\(^\circ\)C) on quiescent elongated cells showing cross-striations using an EPC-7 amplifier (LIST). \(I_{Na}\) was recorded using the whole-cell voltage clamp technique. Ventricular myocytes were superfused with a modified Tyrode’s solution containing (in mmol/L) NaCl 140, KCl 5, MgCl\(_2\) 2.5, CaCl\(_2\) 0.02, HEPES 5, and glucose 5 (pH 7.4 with NaOH). The recording electrode (\(R_e\)) ranged from 0.7 to 1 M\(\Omega\). Capacitive transients were nulled by analog compensation and whole-cell capacitance was estimated from the applied compensation. Series resistance (\(R_s\)) ranged from 3 to 4 M\(\Omega\) and was compensated by 80% to 90%, leaving a voltage error of \(±3\) mV. To avoid the contaminating effect of time-dependent changes in Na\(^+\) channel kinetics, \(I_{Na}\) recordings were made 5 minutes after membrane rupture. Leak currents were typically \(<100\) pA and were not corrected.

Single-channel recordings were performed in the cell-attached configuration using 10-M\(\Omega\) microelectrodes. Cells were depolarized to \(≈5\) mV in a high-K\(^+\) bath containing (in mmol/L) potassium aspartate 130, KCl 10, MgCl\(_2\) 5, 65 \(\mu\)g/mL EGTA 0.05, and glucose 5 (pH 7.4 with KOH). The recording pipette solution contained (in mmol/L) NaCl 140, KCl 5, MgCl\(_2\) 2.5, CaCl\(_2\) 0.02, HEPES 5, and glucose 5 (pH 7.4 with NaOH). Currents were filtered at 2.5 kHz and sampled at 20 kHz.

Action potentials were recorded in the current-clamp configuration. Myocytes were bathed in Tyrode’s solution containing (in mmol/L) NaCl 140, KCl 4, CaCl\(_2\) 2, MgCl\(_2\) 2.5, HEPES 5, and glucose 5 (pH 7.4 with NaOH). The recording electrode (\(R_e\), 2 to 5 M\(\Omega\)) was filled with an internal solution containing (in mmol/L) KCl 140, NaCl 10, EGTA 2, MgCl\(_2\) 1, NaGTP 0.1, MgATP 5, and HEPES 10 (pH 7.2 with KOH). Cells were current clamped (WT, 13±3 pA; ankyrin\(_{b}(+/−)\), 19±3 pA; ankyrin\(_{b}(−/−)\), 16±3 pA; \(P=NS\)) to a resting membrane potential of \(−80\) mV. Action potentials were then elicited by 2-ms depolarizing pulses of twice-diastolic threshold at 1 Hz. APD was measured at 90% repolarization (APD\(_{90}\)).

**Electrocardiography**

Surface ECGs were recorded from 1-day-old mice. Conscious mice were held in the recumbent position with thin adhesive strips on a heating pad at 35\(^\circ\)C. Dry adhesive electrode strips were wrapped around each of their 4 limbs, and ECG leads were connected to an amplifier (Propac) (0.5 to 40 Hz). Lead I consistently resolved ECG waveforms well and was digitized at 500 Hz using a 16-bit analog/digital converter. The recording protocol consisted of a baseline ECG at 35\(^\circ\)C. Subsequent ECGs were recorded at \(≈5\)-minute intervals after transferring the mouse to a room temperature environment that induced heart rate deceleration. To minimize the noise in these low-amplitude ECG waveforms, a signal-averaged ECG (SAECG) was derived from \(≈10\) consecutive, well-defined complexes. The onset of the QRS complex was manually selected as the fiducial point for ensemble averaging using custom software (Testpoint, CEC). The QT interval was measured manually from each SAECG from the beginning of QRS complex to the end of the T wave, defined as the point of return to the isoelectric baseline.

**Data Analysis**

Voltage-clamp whole-cell and single-channel data were compiled and analyzed using custom software written in C language as previously described.\(^8\) Only whole-cell experiments demonstrating adequate voltage control were analyzed as defined by Whalley et al.\(^10\) \(I_{Na}\) density was determined by dividing current amplitude by cell capacitance. For ECG analysis, the rate-corrected QT interval using Bazett’s formula is accurate at the rapid heart rates characteristic of neonatal mice.\(^11\) Therefore, for each mouse, QT intervals were plotted with their corresponding R-R intervals. A semilogarithmic transformation of this plot was best fitted with linear least-squares regression analysis (\(QT=[m\log (R-R)+b]\)). The regression coefficients (\(m\) and \(b\)) were used to calculate the QT interval corresponding to a predetermined R-R interval of 150 ms for each mouse. Continuous variables were compared between WT and ankyrin\(_{b}(+/−)\), as well as WT and ankyrin\(_{b}(−/−)\) using the unpaired Student \(t\) test. Comparisons of continuous variables within groups was done with the paired Student \(t\) test. Data are expressed as mean±SEM. All tests were 2-sided, and differences were considered significant at \(P<0.05\).

**Results**

**Na\(^{+}\) Channel Studies**

Whole-cell capacitance was comparable for WT (18±2 pF, \(n=18\)), ankyrin\(_{b}(+/−)\) (19±2 pF, \(n=15\)), and ankyrin\(_{b}(−/−)\) (16±1 pF, \(n=18\)). Peak \(I_{Na}\) density occurred at a test potential (\(V_t\)) of \(−20\) mV and was similar in WT (444±39 pA/pF) and ankyrin\(_{b}(+/−)\) (347±35 pA/pF). However, in ankyrin\(_{b}(−/−)\), \(I_{Na}\) density was less than in WT (442±57 pA/pF, \(P<0.01\) (Figure 1B)). To evaluate the basis for the reduced \(I_{Na}\) density in ankyrin\(_{b}(−/−)\), cell-attached single-channel recordings were performed serially at \(V_t\) of −20, −40, and −50 mV. Single-channel current was plotted against \(V_t\), and conductance was
determined from the linear regression slope. Single-channel conductance (g) was similar in WT (11.6±0.1 pS, n=7) and ankyrinB(−/−) (10.9±0.1 pS, n=8). Single-channel open probability (P_o) at V_h of −20 mV, determined according to Chandra et al., was also equivalent between ankyrinB(−/−) (0.66±0.03) and WT (0.61±0.1). Because whole-cell current is proportional to g, P_o, and channel number, the lower current density in ankyrinB(−/−) cardiomyocytes was the result of fewer functional Na⁺ channels.

The whole-cell voltage dependence of activation and inactivation are summarized in the Table. No differences in the kinetics of activation or inactivation were apparent between WT and ankyrinB(+/−). When compared with WT, ankyrinB(−/−) had a hyperpolarizing shift in V_0.5 for activation (−49.2±0.9 [n=18] versus −46.3±0.9 mV [n=18], P<0.05) (Figure 1C). The V_0.5 for inactivation was also more negative in ankyrinB(−/−) (−79.5±1.4 mV, n=11) than in WT (−74.6±1.6 mV [n=12], P<0.05) (Figure 2). No accompanying differences in slope factors were found. To determine the basis for the inactivation shift in ankyrinB(−/−), recovery from inactivation kinetics was assessed using a 2-pulse protocol with varying recovery intervals between the pulses at a holding potential (V_h) of −100 mV (Figure 3). At −100 mV, the rate of development of inactivation approximates 0, which permits recovery rates from inactivation to be accurately determined. Recovery kinetics were fit with a biexponential function, giving a slower rate of recovery from inactivation in ankyrinB(−/−) when compared with WT (Table), which accounted for the hyperpolarizing shift in V_0.5 for inactivation in ankyrinB(−/−).

To resolve the macroscopic kinetic differences between WT and ankyrinB(−/−), cell-attached single-channel studies were performed. For ankyrinB(−/−), patches with only 1 to 2 channels were readily obtained at a V_h of −100 mV. In contrast, WT patches frequently had multiple channels at V_h of −100 mV and required more depolarization V_h to resolve single-channel events. This behavior is consistent with our findings of reduced I_Na density in ankyrinB(−/−) as a result of lower channel number. Representative single-channel recordings from 10 consecutive depolarizing steps at −20, −40, and −50 mV are shown in Figure 4 for WT (2-channel patch) and ankyrinB(−/−) (1-channel patch). Despite fewer channels in the ankyrinB(−/−) patch, the ensemble average currents at V_h of −40 and −50 mV were larger in ankyrinB(−/−), suggesting a hyperpolarizing shift in activation, which is consistent with the whole-cell studies. In ankyrinB(−/−) and WT patches, mean open times were voltage

![Figure 2](image1.png)  
**Figure 2.** A. Representative whole-cell I_Na in WT and ankyrinB(−/−). I_Na was recorded with the inactivation protocol shown in the inset at conditioning potentials of −130, −90, −80, −70, −60, and −45 mV. B. Availability-voltage relationship for panel A. Slope factors were 6.7 mV (WT), 5.6 mV (ankyrinB(+/−)), and 5.7 mV (ankyrinB(−/−)).

![Figure 3](image2.png)  
**Figure 3.** A. Representative whole-cell I_Na in WT and ankyrinB(−/−). I_Na was recorded with the recovery from inactivation protocol shown in the inset. B. Normalized current-recovery interval relationship for panel A. The amplitude and time constants for the slow and fast components of recovery from inactivation were A_s = −0.31, τ_s = 20 ms, A_f = 0.73, and τ_f = 7.0 ms (WT); A_s = −0.20, τ_s = 35 ms, A_f = 0.80, and τ_f = 8.9 ms (ankyrinB(+/−)); and A_s = 0.63, τ_s = 28 ms, A_f = 0.38, and τ_f = 8.7 ms (ankyrinB(−/−)).

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<table>
<thead>
<tr>
<th>Comparison of Whole-Cell Na⁺ Channel Kinetics</th>
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<tbody>
<tr>
<td>Activity</td>
<td>Wild-Type (n)</td>
<td>AnkyrinB(+/−) (n)</td>
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<tr>
<td>V_0.5, mV</td>
<td>−46.3±0.9 (18)</td>
<td>−46.7±0.8 (15)</td>
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<tr>
<td>k_o, mV</td>
<td>8.4±0.2</td>
<td>8.0±0.2</td>
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<tr>
<td>Inactivation</td>
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<td></td>
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<tr>
<td>V_0.5, mV</td>
<td>−74.6±1.6 (12)</td>
<td>−77.0±1.2 (10)</td>
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<tr>
<td>k_o, mV</td>
<td>6.8±0.3</td>
<td>6.7±0.4</td>
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<tr>
<td>Recovery from inactivation</td>
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<td></td>
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<tr>
<td>A_inf, ms</td>
<td>−0.29±0.07 (5)</td>
<td>−0.24±0.1 (3)</td>
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<tr>
<td>τ_inf, 100 ms, ms</td>
<td>28±9</td>
<td>31±4</td>
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<td>A_90, ms</td>
<td>−0.76±0.08</td>
<td>−0.82±0.12</td>
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<tr>
<td>τ_90, 100 ms, ms</td>
<td>6.8±0.9</td>
<td>7.8±0.7</td>
</tr>
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*P<0.05 vs WT.
dependent and were 2-fold longer in ankyrinB(−/−) at V1 of −40 and −50 mV (Figure 5A). Open-time histograms were fit with a monoexponential function, suggesting a single open state.12 As expected from a Poisson process, the time constants for channel closing (τclosing) derived from these histograms and the mean open times were the same (Figure 5B). In ankyrinB(−/−), more frequent reopenings were seen at V1 of −40 and −50 mV when compared with WT. To characterize these reopening and mean open times were the same (Figure 5B). In ankyrin B (−/−) (1-channel patch, No. 090299gij1). Figure 5. A, Mean open-time–voltage relationship in WT and ankyrinB(−/−) for several patches. B, Closing time constant (τclosing)–voltage relationship for several patches. Both mean open times and τclosing were greater in ankyrinB(−/−) at V1 of −50 and −40 mV compared with WT.

fore not comparable with WT. At −50 mV, frequent reopenings in ankyrinB(−/−) permitted closed-time histograms to be constructed in 6 patches, 3 of which were fit with a biexponential function (τfast=7.2±1.5 ms, τslow=0.26±0.05 ms).

Channel reopenings in ankyrinB(−/−) were observed late into the depolarizing test pulse at V1 of −40 and −50 mV. During whole-cell INa relaxation or decay (iNa-relax), channel reopenings have been described at these hyperpolarizing test potentials.12,13 To identify late channel reopenings beyond the time course of iNa-relax, channel openings were detected after a time interval (T) corresponding to 5 times the time constant for iNa-relax (τrelax). τrelax was derived from a monoexponential fit of iNa-relax and was longer in WT than ankyrinB(−/−) at V1 of −40 (4.4±0.4 [n=18] versus 3.3±0.2 ms [n=18], P<0.05) and −50 mV (10±1 versus 7.4±0.6 ms, P<0.05). τrelax for WT was therefore also applied to ankyrinB(−/−) in calculating T. Late channel openings were defined as single-channel openings after 25 ms at V1 of −40 mV and after 50 ms at V1 of −50 mV for each 100-ms test depolarization. The integrated current from each depolarization was divided by the single-channel current amplitude and the number of channels in the patch to give the single-channel open probability (Po).

A diary of late events was generated by plotting Po versus trace number for 200 successive depolarizations. In all 7 WT patches, late reopenings were infrequent and occurred at widely spaced intervals as small spikes in the Po diary. In contrast, 3 of the 8 ankyrinB(−/−) patches showed significantly more late activity that was distributed throughout the Po diary at V1 of −40 and −50 mV (Figure 7).

**Action Potential Measurements**

To determine the effect of late iNa on ventricular repolarization, action potentials were recorded from single myocytes (Figure 8). At a stimulation frequency of 1 Hz, APD90,s were comparable in WT (274±22 ms [n=9]) and ankyrinB(+/−) (283±14 ms [n=9]). However, ankyrinB(−/−) had longer APD90 than WT (354±26 ms [n=8], P<0.05).

**ECG Characteristics**

Standard bipolar lead I was recorded from 1-day-old mice. Their body weights differed slightly, as follows: WT,
probability of a single channel opening after 25 ms at depolarizing trials. Late opening probability was defined as the probability of a single channel opening after 25 ms at V_{m} of -40 mV and after 50 ms at V_{m} of -50 mV. Late opening probability was greater in ankyrin_{B(-/-)} at both V_{m} compared with WT.

1.53±0.16 g (n=14); ankyrin_{B(+/-)}, 1.33±0.15 g (n=8, P=0.05 versus WT); and ankyrin_{B(-/-)}, 1.26±0.03 g (n=12, P<0.01 versus WT). Three ankyrin_{B(-/-)} mice were excluded because of marked heart rate variability at 35°C, which made QT-interval measurements unreliable. No such heart rate variability was observed in WT or ankyrin_{B(+/-)} mice. Representative SAECG traces are shown in Figure 9. The T-wave morphology was similar in the 3 genotypes and characterized by a monophasic, upright T wave. Baseline heart rate at 35°C was similar between WT (434±13 bpm) and ankyrin_{B(+/-)} (424±8 bpm) mice but was lower in ankyrin_{B(-/-)} than WT (380±14 bpm, P<0.01) mice. The average decline in heart rate at room temperature over a ~20-minute recording period was similar among WT (194±12 bpm), ankyrin_{B(+/-)} (185±8 bpm), and ankyrin_{B(-/-)} (172±14 bpm) mice. The QT interval at an R-R interval of 150 ms, which corresponded to a physiological heart rate of 400 bpm, was comparable in WT (83±2 ms), ankyrin_{B(+/-)} (86±1 ms), and ankyrin_{B(-/-)} (81±5 ms) mice. The slope of the QT–log R-R linear relationship was also similar between WT (150±7) and ankyrin_{B(+/-)} (167±18) mice. However, ankyrin_{B(-/-)} mice showed greater QT prolongation in response to R-R interval lengthening, as suggested by a larger regression slope when compared with WT (202±22, P<0.05). Over a 150-ms change in cycle length from 150 to 300 ms, the QT interval increased by 61±7 ms in ankyrin_{B(-/-)} compared with 45±2 ms in WT (P<0.05) mice.

At physiological heart rates, 2 ankyrin_{B(-/-)} mice exhibited intermittent failure of AV conduction, resulting in P waves without corresponding QRS complexes, which resolved at lower heart rates. Heart block was not apparent in any WT or ankyrin_{B(+/-)} mouse. No ventricular arrhythmias were observed in the 3 genotypes.

Discussion

In the present study, neonatal ankyrin_{B(-/-)} ventricular myocytes exhibited several abnormalities in the Na^{+} channel compared with WT, including the following: (1) reduced I_{Na} density due to fewer functional Na^{+} channels; (2) hyperpolarizing shift in voltage-dependent activation and inactivation, the latter due to slower recovery from inactivation; and (3) greater late I_{Na} due to longer channel openings and late reopenings. Greater late I_{Na} may contribute to longer APD_{90} in ankyrin_{B(-/-)} cardiomyocytes. Although prolonged cardiomyocyte repolarization is not associated with QT-interval lengthening at physiological heart rates, QT-rate adaptation is abnormal in ankyrin_{B(-/-)} mice when compared with WT, resulting in greater QT prolongation with heart rate deceleration. In contrast, ankyrin_{B(+/-)} mice have similar Na^{+} channel properties, APD_{90} and QT adaptation to WT, despite reduced cardiac ankyrin_{B} levels.9

The phenotypic abnormalities of neonatal ankyrin_{B(-/-)} mice have been well described and include hypoplasia of select regions of the nervous system9 and a skeletal muscle myopathy.1 These derangements may contribute to the reduced life span of ankyrin_{B(-/-)} mice, with more than half
of the animals dying on the first postnatal day. Although knockout mice have smaller hearts, cardiomyopathy is not evident. In contrast, ankyrin(−/−) mice are similar to WT with respect to body weight, heart size, and survival (S. Tuvia, V. Bennett, unpublished observations, 1999). In the present study, 1-day-old mice were used to permit comparisons among the 3 genotypes. The neonatal period is characterized by cardiac Na⁺ channel maturation due to postnatal sympathetic innervation of the heart. In addition, developmental changes in potassium currents contribute to shorter APD in the adult when compared with the neonatal mouse. Thus, the differences in I物联网 and QT intervals between neonatal ankyrin(−/−) and WT mice may not be generalizable to the adult phenotypes. Despite this limitation, the present study is the first to characterize the effect of ankyrinB deficiency on the cardiac Na⁺ channel and ventricular repolarization in vivo.

**Basis for Abnormal Na⁺ Channel Properties**

Modulation of Na⁺ channel function by the cytoskeleton has been previously described. In particular, disruption of the actin-based cytoskeleton in ventricular myocytes with cytochalasin D alters I物联网 and Na⁺ channel kinetics. Whole-cell I物联网 and Na⁺ channel conductance is reduced without a change in single-channel conductance. In addition, late Na⁺ channel burst openings develop at hyperpolarizing test potentials. Greater Na⁺ channel activation has also been induced in epithelial and leukemic cells after actin depolymerization with cytochalasin D or gelsolin.

AnkyrinB localizes to the sarcolemma in a costamere pattern. The lack of ankyrinB at these membrane sites may cause incorrect organization of the costamere and the cytoskeletal elements known to bind to it, such as spectrin, dystrophin, and vinculin. Because costameres attach the sarcolemma to the sarcomeres, ankyrinB deficiency may disrupt folding of the sarcolemma during myocyte contraction. In view of this, we speculate that ankyrinB(−/−) cardiomyocytes have abnormal arrangement of their cytoskeleton, which may contribute to fewer functional Na⁺ channels with altered kinetics.

**Late I物联网 and Cardiac Repolarization**

Late I物联网 can prolong cardiac repolarization, as is evident from the inactivation-deficient Na⁺ channels responsible for LQT3. Unlike the LQT3-mutant Na⁺ channel, which undergoes late reopenings at plateau potentials of −20 mV, the late Na⁺ channel reopenings in ankyrin(−/−) are seen only at more hyperpolarized potentials of −40 and −50 mV. At these potentials, no significant inward current normally competes with the delayed and inward rectifying currents. Thus, late I物联网 may still prolong phase 3 of the action potential in ankyrin(−/−). However, differences in other ionic currents may also play a role in lengthening cardiac repolarization in ankyrin(−/−).

Despite longer APD物联网 at 1 Hz stimulation in ankyrin(−/−) cardiomyocytes, their QT interval at physiological heart rates is similar to that of WT. The discrepancy in APD物联网 and QT-interval response may be due to differences in cycle length. Cardiac repolarization time is dependent on cycle length, which will influence the contribution of abnormal repolarizing currents to the action potential. In ankyrin(−/−), late I物联网 may have less influence on APD and QT interval at physiological heart rates at which potassium repolarizing currents such as I钾 accumulate. With less I钾 at lower heart rates, late I物联网 may predominate enough to prolong APD and the QT interval in the knockout mouse. This hypothesis is supported by longer QT intervals in ankyrin(−/−) at lower heart rates when compared with WT.

**Clinical Correlation With LQTS**

LQTS is a genetically heterogeneous condition characterized by QT-interval prolongation due to well-defined mutations in the K channel (LQT1, LQT2, and LQT5) or Na⁺ channel (LQT3). In contrast to LQT1, LQT2, LQT3, and LQT5, LQT4 is characterized by marked QT-interval prolongation and sinus bradycardia. The genetic defect in LQT4 has not yet been defined, although it has been localized to 4q25 to 27. AnkyrinB(−/−) mice display several ECG features of LQTS, including bradycardia, incomplete AV block, and abnormal QT-rate adaptation, the latter due in part to their late I物联网. The basis for the slower heart rates in ankyrinB(−/−), as well as functional incomplete heart block in a few knockout mice, is not clear but may relate to abnormalities in other ionic currents such as pacemaker currents and I钾. The gene for ankyrinB (ANK2) has been localized to 4q25 to 27, which is also shared with LQT4. We therefore speculate that ankyrinB deficiency may contribute to LQTS, in particular LQT4. In previously described transgenic murine models of LQTS involving loss of potassium channel function, several similarities are seen with ankyrinB(−/−) mice, including APD prolongation and normal QT intervals at physiological heart rates, and abnormal QT-rate adaptation.

Unlike ankyrinB(−/−), the neonatal ankyrinB(+/−) mice have less cardiac ankyrinB deficiency and do not share the same ECG abnormalities. In contrast, adult LQT4 patients manifest the disease as heterozygote gene carriers as a result of autosomal dominant inheritance. It is possible that developmental maturation of cardiac ion channels, including the Na⁺ channel, may unmask repolarization abnormalities in adult ankyrinB(+/−) that are not apparent in the neonatal mice. It will be of interest to examine adult ankyrinB(+/−) mice for bradycardia and repolarization abnormalities of LQT4.

In conclusion, ankyrinB deficiency alters Na⁺ channel function and prolongs cardiac repolarization in neonatal ankyrinB(−/−) mice. A plausible mechanism for these changes may relate to cardiomyocyte cytoskeletal disruption. Future genetic testing of LQT4 patients for ANK2 mutations is warranted to further define the genetic basis for this condition.

**Acknowledgments**

This study was supported in part by NIH Grant HL-32708 (to A.O.G.). V.S.C. is a research fellow of the Heart and Stroke Foundation of Canada.

**References**


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*Circ Res.* 2000;86:441-447
doi: 10.1161/01.RES.86.4.441

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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