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Defining new insight into atypical arrhythmia: a computational model of ankyrin-B syndrome

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Wolf RM, Mitchell CC, Christensen MD, Mohler PJ, Hund TJ. Defining new insight into atypical arrhythmia: a computational model of ankyrin-B syndrome. *Am J Physiol Heart Circ Physiol* 299: H1505–H1514, 2010. First published August 20, 2010; doi:10.1152/ajpheart.00503.2010.— Normal cardiac excitability depends on the coordinated activity of specific ion channels and transporters within specialized domains at the plasma membrane and sarcoplasmic reticulum. Ion channel dysfunction due to congenital or acquired defects has been linked to human cardiac arrhythmia. More recently, defects in ion channel-associated proteins have been associated with arrhythmia. Ankyrin-B is a multifunctional adapter protein responsible for targeting select ion channels, transporters, cytoskeletal proteins, and signaling molecules in excitable cells, including neurons, pancreatic β-cells, and cardiomyocytes. Ankyrin-B dysfunction has been linked to cardiac arrhythmia in human patients and ankyrin-B heterozygous (ankyrin-B$^{+/-}$) mice with a phenotype characterized by sinus node dysfunction, susceptibility to ventricular arrhythmias, and sudden death ("ankyrin-B syndrome"). At the cellular level, ankyrin-B$^{+/-}$ cells have defects in the expression and membrane localization of the Na^+/Ca^{2+} exchanger and Na^+-K^+-ATPase, Ca^{2+} overload, and frequent afterdepolarizations, which likely serve as triggers for lethal cardiac arrhythmias. Despite knowledge gathered from mouse models and human patients, the molecular mechanism responsible for cardiac arrhythmias in the setting of ankyrin-B dysfunction remains unclear. Here, we use mathematical modeling to provide new insights into the cellular pathways responsible for Ca^{2+} overload and afterdepolarizations in ankyrin-B$^{+/-}$ cells. We show that the Na^+/Ca^{2+} exchanger and Na^+-K^+-ATPase play related, yet distinct, roles in intracellular Ca^{2+} accumulation, sarcoplasmic reticulum Ca^{2+} overload, and afterdepolarization generation in ankyrin-B$^{+/-}$ cells. These findings provide important insights into the molecular mechanisms underlying a human disease and are relevant for acquired human arrhythmia, where ankyrin-B dysfunction has recently been identified.

Na^+/Ca^{2+} exchanger; Na^+-K^+-ATPase; mathematical model; trafficking; calcium

NORMAL CARDIAC EXCITABILITY depends on the coordinated biophysical activity of specific ion channels and transporters on the plasma membrane. Over the past decade, gene mutations that affect ion channel biophysical activity have been linked with fatal human arrhythmias (19). Recently, a new class of gene mutations has been identified that alters the local coupling of ion channels with cytoskeletal and regulatory proteins (5, 20, 22, 27, 28, 36, 41, 42). Based in part on these important findings, it is now clear that ion channel and transporter function depend on normal biophysical properties and proper local membrane localization/organization.

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Ankyrin polypeptides are responsible for the targeting and stabilization of ion channels, transporters, and signaling molecules at cell membranes of diverse cell types, including cardiomyocytes, neurons, pancreatic β-cells, and erythrocytes (12). Over the past decade, members of the ankyrin family (ankyrin-R, ankyrin-B, and ankyrin-G) have been linked to a number of human diseases, including hereditary spherocytosis, cardiac arrhythmia, diabetes, and muscular dystrophy (1, 8, 16, 27, 28). Ankyrin-B dysfunction has been linked to both congenital and acquired arrhythmias (15, 17, 23, 26, 28, 35). In fact, nine loss-of-function variants in *ANK2* (the gene encoding ankyrin-B) have been identified in human patients with a complex cardiac arrhythmia syndrome ("long QT 4" or "ankyrin-B syndrome") characterized by sinus node dysfunction, increased susceptibility to ventricular arrhythmias, and sudden death under stress (17, 26, 28, 29, 34). Ankyrin-B-deficient (ankyrin-B$^{+/-}$) mice display an increased susceptibility to stress-induced arrhythmias and sudden death, similar to human patients (23, 26, 28). Importantly, ankyrin-B$^{+/-}$ mice show defects in ion channel and transporter targeting, abnormal Ca^{2+} homeostasis, and an increased likelihood of potentially life-threatening afterdepolarizations (28). In ventricular cardiomyocytes, ankyrin-B is responsible for the proper membrane localization and function of Na^+-K^+-ATPase (NKA), Na^+/Ca^{2+} exchanger (NCX), inositol 1,4,5-trisphosphate ($InsP_3$) receptor, and protein phosphatase 2A (PP2A) (2, 7, 29). Despite the wealth of information gathered from human patients and the mouse model of ankyrin-B syndrome, the direct link between specific membrane protein changes and cardiac arrhythmia has yet to be established.

Mathematical modeling of excitable cells has been used to generate important insights into the cellular mechanisms underlying a wide variety of human diseases, including cardiac arrhythmia, epilepsy, and diabetes (6, 14, 16, 32). In this study, we used mathematical modeling to identify the cellular pathway responsible for abnormal Ca^{2+} handling and cardiac arrhythmia in ankyrin-B$^{+/-}$ cells. Our computer simulations showed that loss of NCX and NKA membrane targeting in ankyrin-B$^{+/-}$ cells resulted in accumulation of intracellular Na^+ and Ca^{2+} under basal conditions. Furthermore, ankyrin-B reduction predisposed the cell to Ca^{2+} overload, frequent spontaneous Ca^{2+} release, and action potential (AP) afterdepolarizations during rapid pacing in the presence of isoproterenol. While loss of NKA function contributed to the increased Ca^{2+} transients in ankyrin-B$^{+/-}$ cardiomyocytes, abnormal NCX targeting was the dominant mechanism for Ca^{2+} overload in the sarcoplasmic reticulum (SR), spontaneous Ca^{2+} release, and afterdepolarizations. These findings provide important insights into the molecular mechanism underlying a

human disease and highlight the related, yet distinct, roles of NCX and NKA in ventricular cardiomyocytes. Considering the association between ankyrin-B dysfunction and acquired arrhythmia as well as arrhythmia susceptibility in the general population (15, 35), we expect the insights generated by this study to have broader relevance for human disease.

METHODS

Mathematical model of the ankyrin-B+/− cardiomyocyte. The mathematical model used in this study was based on a model of the murine ventricular AP (3, 4) since much of the experimental data came from the mouse. Importantly, the formulations for NKA and NCX current have been well validated against experimental data from mammalian ventricular myocytes (21) (Supplemental Material, Supplemental Fig. S1).[1] Simulations were also performed using a well-validated model of the human ventricular myocyte (38). Modifications to the equations were made to account for experimentally measured changes in NCX and NKA membrane expression in ankyrin-B$^{+/-}$ cardiomyocytes (Fig. 1, *A–E*) (23, 25, 28, 29). Specifically, NCX was scaled to produce a 40% reduction in current at a test potential of −10 mV compared with control (wild type), consistent

[1]Supplemental Material for this article is available online at the *American Journal of Physiology-Heart and Circulatory Physiology* website.

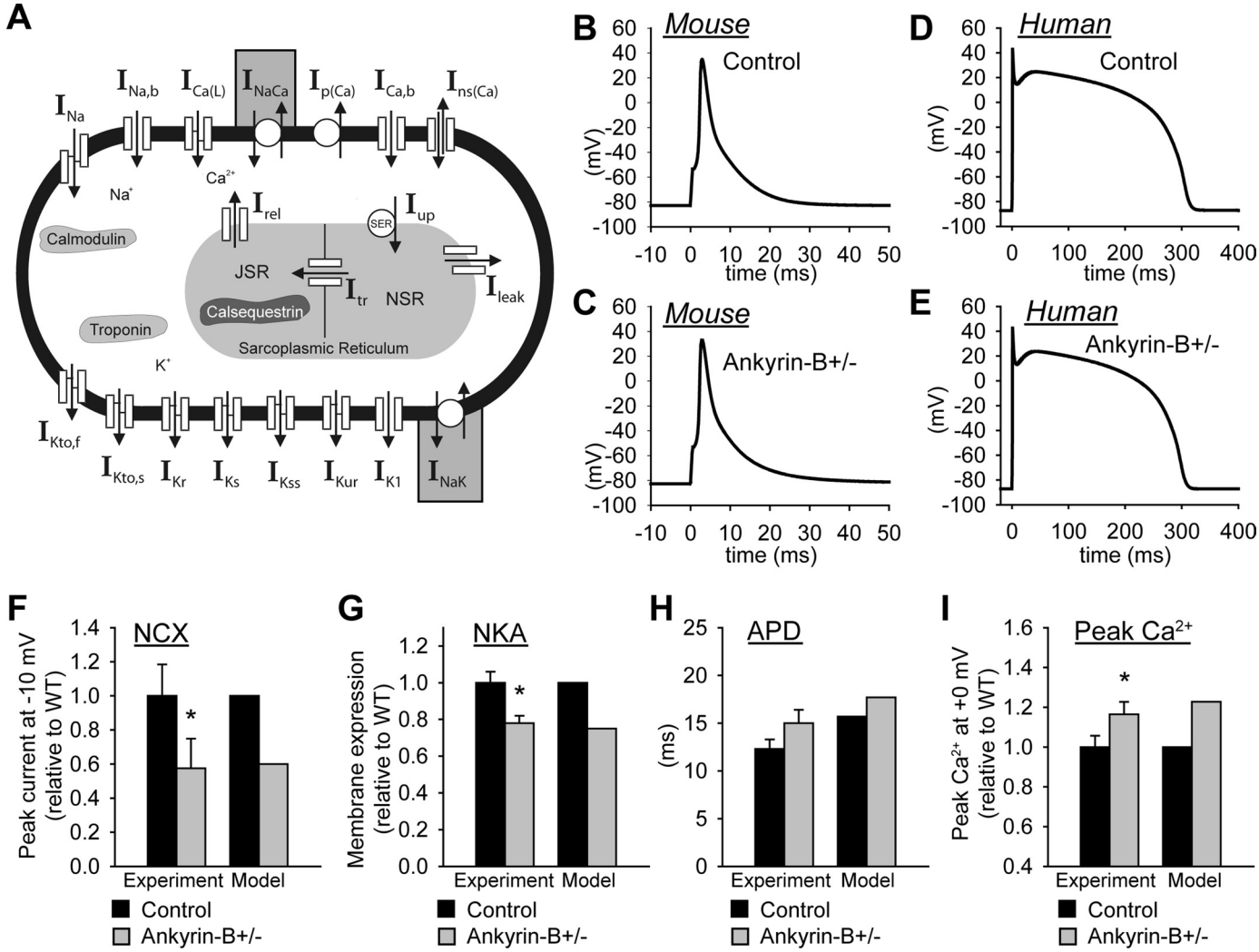


Fig. 1. Mathematical model of the ankyrin-B-deficient (ankyrin-B$^{+/-}$) cell. *A*: schematic of the mouse ventricular cell model. Na^+/Ca^{2+} exchanger (NCX) current (I_{NaCa}) and Na^+-K^+-ATPase (NKA) current (I_{NaK}) were altered in the model of the ankyrin-B$^{+/-}$ cell (shaded boxes). I_{Na}, Na^+ current; $I_{Na,b}$, background Na^+ current; I_{CaL}, L-type Ca^{2+} current; $I_{p(Ca)}$, sarcolemmal Ca^{2+} pump; $I_{Ca,b}$, background Ca^{2+} current; $I_{ns(Ca)}$, nonspecific Ca^{2+} current; $I_{Kto,f}$, fast component of the transient outward K^+ current; $I_{Kto,s}$, slow component of the transient outward K^+ current; I_{Kr}, fast component of the delayed recifier K^+ current; I_{Ks}, slow component of the delayed recifier K^+ current; I_{Kss}, steady-state K^+ current; I_{Kur}, ultrarapid K^+ current; I_{K1}, inward rectifier K^+ current; I_{rel}, Ca^{2+} release flux; I_{up}, Ca^{2+} uptake flux; SER, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; JSR, junctional sarcoplasmic reticulum (SR); NSR, network SR; I_{tr}, Ca^{2+} transfer flux; I_{leak}, Ca^{2+} leak flux. *B–E*: simulated action potentials (APs) in control (*B* and *D*) and ankyrin-B$^{+/-}$ (*C* and *E*) cardiomyocytes from the mouse (*B* and *C*) and human (*D* and *E*) ventricular cell models [10th action potential (AP) shown at a cycle length (CL) of 1,000 ms]. *F*: simulated I_{NaCa} at a test potential of −10 mV from wild-type cell and an ankyrin-B$^{+/-}$ cell compared with experimental measurements (n = 12, *P < 0.05) (17). In both the simulation and experiment, intracellular Na^+ concentration = 20 mM, extracellular Na^+ concentration = 145 mM, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) = 1 μM, and extracellular Ca^{2+} concentration = 2 mM. *G*: simulated and experimentally measured (n = 3, *P < 0.01) (25) NKA membrane expression in wild-type and ankyrin-B$^{+/-}$ mouse ventricular cells. *H*: simulated and experimentally measured (n = 17, P = not significant) (28) AP duration (APD) at 90% repolarization. *I*: simulated and experimentally measured (n = 18, *P < 0.001) peak Ca^{2+} transients. In both the simulation and experiment, the cell was pulsed four times to test potential of +0 mV from a holding potential of −40 mV (28).

with experimental measurements from adult ankyrin-B$^{+/-}$ myocytes (17) (Fig. 1*F*). Also, the maximum NKA current was decreased 25% based on experimental measurements showing reduced NKA surface expression in ankyrin-B$^{+/-}$ myocytes (as assessed by [^{3}H]ouabain binding) (25) (Fig. 1*G*). The peak L-type Ca^{2+} current (I_{CaL})-voltage relationship was no different between the control and ankyrin-B$^{+/-}$ cell models (not shown), consistent with experimental measurements in ventricular myocytes (28). Importantly, simulated APs and Ca^{2+} transients showed good agreement with experimental measurements (28) (Fig. 1, *H* and *I*). The complete equations and parameters for the models used in this study may be found in the Supplemental Material.

Mathematical model of isoproterenol effects. The effects of the β-adrenergic receptor agonist isoproterenol (saturating concentration ≥ 0.1 μM) on the AP and Ca^{2+} transient were simulated according to previously published formulations (9, 40). Specifically, isoproterenol effects on I_{CaL}, the slow component of the delayed rectifier K^+ current (I_{Ks}), and sarco(endo)plasmic reticulum Ca^{2+}-ATPase current (I_{up}) were incorporated into the models to simulate β-adrenergic stimulation (Supplemental Material) (9, 40). The modified equations and parameters may be found in the Supplemental Material.

Pacing protocol. Models were paced from rest to steady state over a range of pacing cycle lengths (CLs; from 2,000 to 200 ms, stimulus amplitude = −60 μA/μF for the mouse and −52 μA/μF for the human; stimulus duration = 0.5 ms for the mouse and 1 ms for the human).

RESULTS

Ankyrin-B deficiency promotes Ca^{2+} overload at baseline. To determine the electrophysiological consequences of abnormal NCX and NKA membrane expression in ankyrin-B$^{+/-}$ cardiomyocytes, we paced the control and ankyrin-B$^{+/-}$ cell models (CL = 1,000 ms; Fig. 2). Consistent with experimental data (28), the simulated AP from the ankyrin-B$^{+/-}$ mouse myocyte was slightly longer than the control AP (Figs. 1*H* and

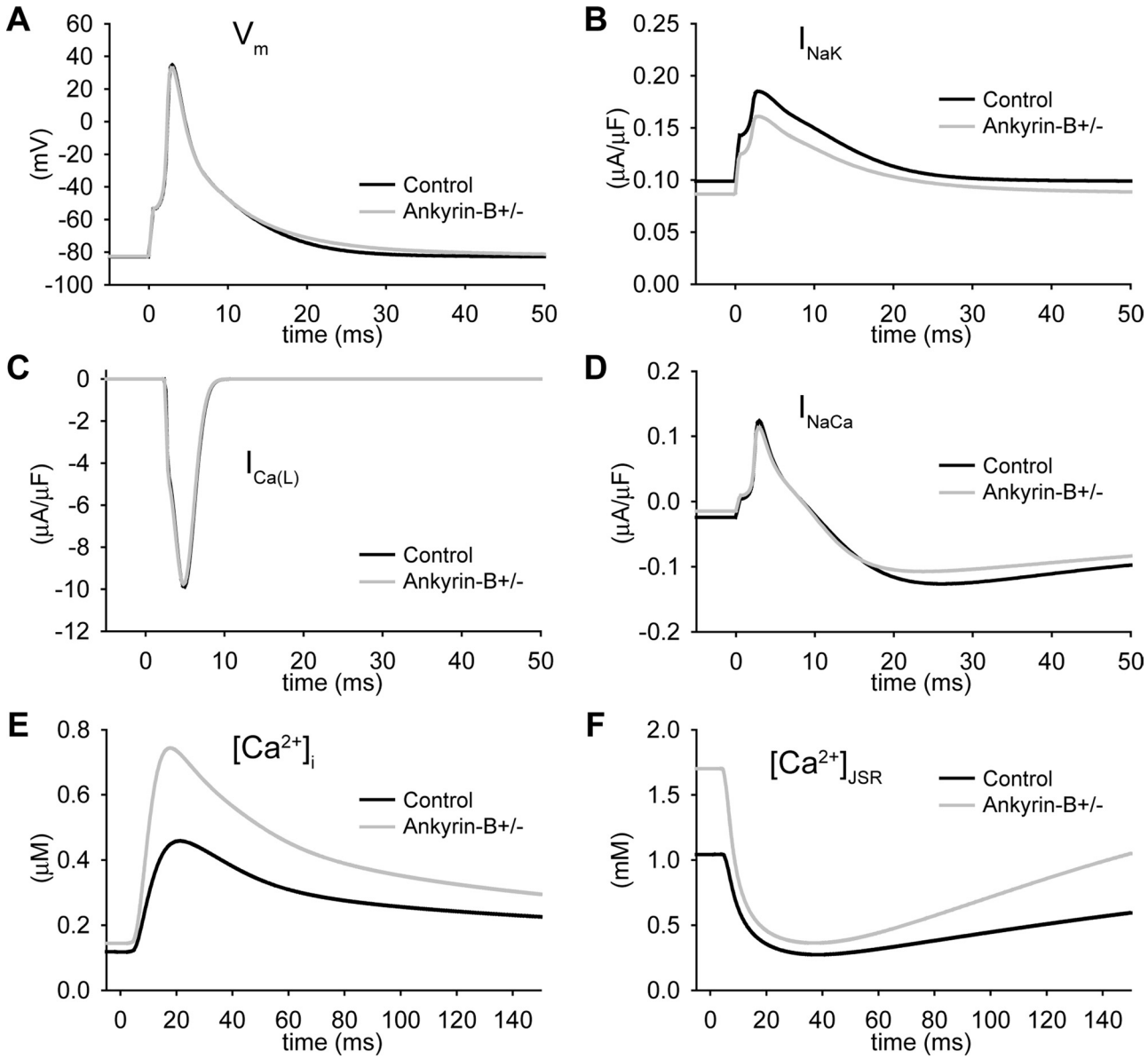


Fig. 2. Ca^{2+} accumulation at baseline in the ankyrin-B$^{+/-}$ cell. *A–F*: simulated steady-state AP (*A*), I_{NaK} (*B*), I_{CaL} (*C*), I_{NaCa} (*D*), Ca^{2+} transient (*E*), and JSR Ca^{2+} concentration ($[Ca^{2+}]_{JSR}$; *F*) in control and ankyrin-B$^{+/-}$ mouse ventricular cells (CL = 1,000 ms). V_m, membrane potential.

Table 1. *Intracellular Na^+ concentration in a computational model of ankyrin-B syndrome*

Cycle Length	Control	Ankyrin-B Deficient	Na^+/Ca^{2+} Exchanger Deficient	Na^+-K^+-ATPase Deficient
Rest				
Without Iso	11.84	13.72	11.53	14.03
200 ms				
Without Iso	12.3	14.24	12.01	14.53
With Iso	12.25	14.18	11.96	14.48
1,000 ms				
Without Iso	11.95	13.85	11.64	14.15
With Iso	11.88	13.79	11.58	14.09

Values are in mM. Iso, isoproterenol.

2*A*). Whereas the AP morphology and AP duration (APD) were similar between the ankyrin-$B^{+/-}$ and control model, loss of NCX and NKA targeting led to dramatic Ca^{2+} and Na^+ accumulation in the cytosol and SR of the ankyrin-$B^{+/-}$ cell (Fig. 2, *E* and *F*, and Table 1). Specifically, ankyrin-B-deficient cells displayed an increase in intracellular Na^+ concentration (Table 1), Ca^{2+} concentration in the junctional SR ($[Ca^{2+}]_{JSR}$), and Ca^{2+} transient amplitude (Fig. 2, *E* and *F*), consistent with experimental measurements from ankyrin-$B^{+/-}$ mice (28). Ca^{2+} bound to SR calsequestrin ($[Ca^{2+}$-CSQN]) was also increased in ankyrin-$B^{+/-}$ cells but remained below the threshold for eliciting spontaneous Ca^{2+} release (not shown).

Arrhythmias in ankyrin-$B^{+/-}$ mice and human patients with ankyrin-B syndrome are commonly observed with increased heart rate (e.g., after stress or exercise) (28). To determine whether loss of NCX and NKA altered the response of the ankyrin-$B^{+/-}$ myocyte to changes in pacing rate, the ankyrin-$B^{+/-}$ and control models were paced over a CL range from 2,000 to 200 ms. APD in the ankyrin-$B^{+/-}$ model was slightly longer than control at all pacing CLs (Fig. 3*A*). Ca^{2+} transient amplitude, $[Ca^{2+}]_{JSR}$, and $[Ca^{2+}$-CSQN] were also greater at every CL (Fig. 3, *B–D*). Consequently, the levels of Ca^{2+} bound to troponin were greater in ankyrin-$B^{+/-}$ cells compared with control (not shown), consistent with increased contractility at baseline (absence of β-adrenergic stimulation) in ankyrin-B-deficient myocytes (25). Importantly, spontaneous release was not observed in the ankyrin-$B^{+/-}$ cell even during rapid pacing (CL = 200 ms; not shown). Spontaneous release was not observed during rapid pacing in the human model either (CL = 500 ms; not shown). These results indicate that while loss of NCX and NKA targeting promoted Ca^{2+} accumulation in ankyrin-$B^{+/-}$ cells, it was not sufficient to trigger spontaneous release under basal conditions (absence of β-adrenergic stimulation).

Role of NCX and NKA in Ca^{2+} overload in ankyrin-$B^{+/-}$ cells. To identify the molecular mechanism responsible for Ca^{2+} accumulation in ankyrin-$B^{+/-}$ cells, we determined APs and Ca^{2+} transients in the ankyrin-$B^{+/-}$ cell with NKA (Fig. 4, NCX deficient, red lines) or NCX restored to normal levels (Fig. 4, NKA deficient, blue lines). Restoring normal NCX targeting had little impact on APD (compare ankyrin-B and NKA deficient in Fig. 4, *A* and *D*; CL = 1,000 ms) in the

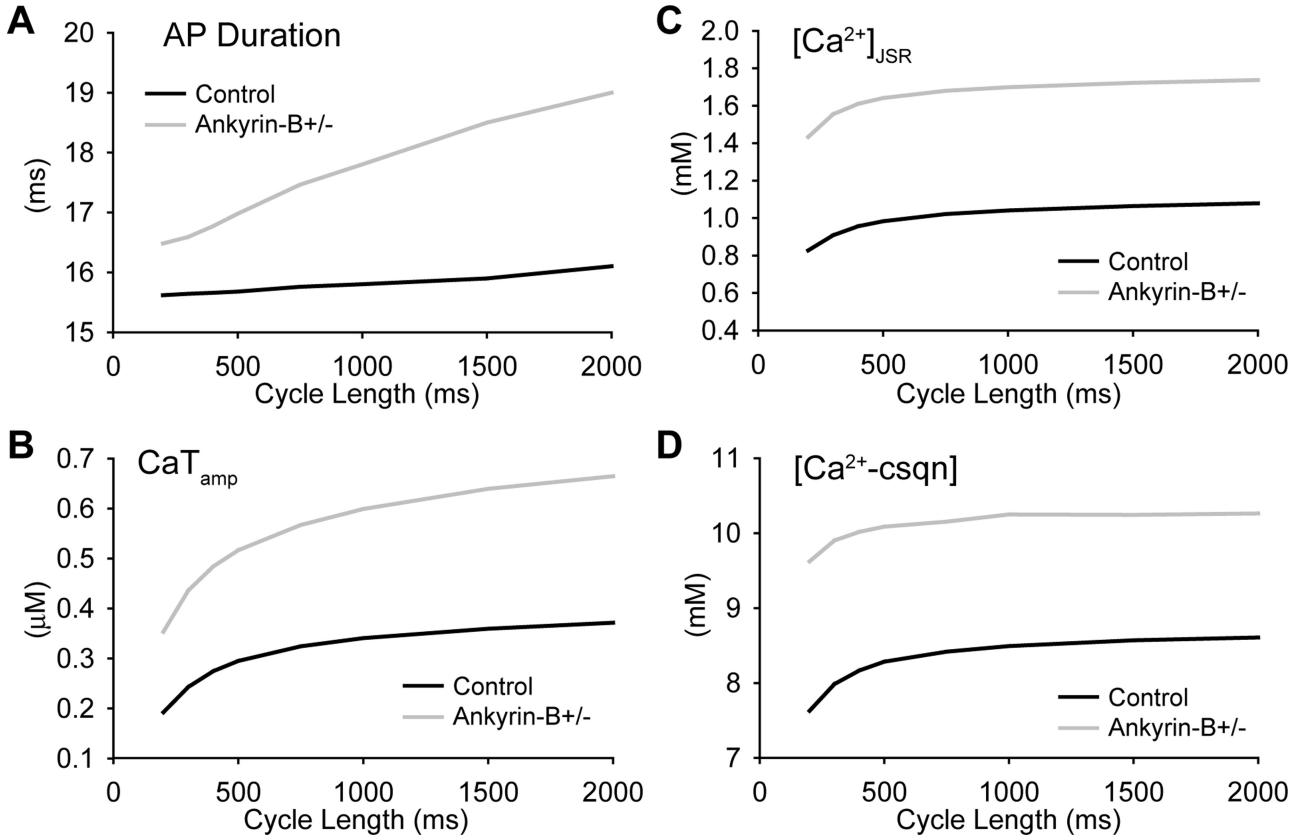


Fig. 3. Rate dependence of the AP and Ca^{2+} transient in wild-type and ankyrin-$B^{+/-}$ cells. *A–D*: APD (*A*), Ca^{2+} transient amplitude (CaT_{amp}; *B*), $[Ca^{2+}]_{JSR}$ (*C*), and concentration of Ca^{2+} bound to calsequestrin ($[Ca^{2+}$-CSQN]; *D*) in control and ankyrin-$B^{+/-}$ cells paced to steady state at CLs of 200, 400, 500, 750, 1,000, 1,500, and 2,000 ms.

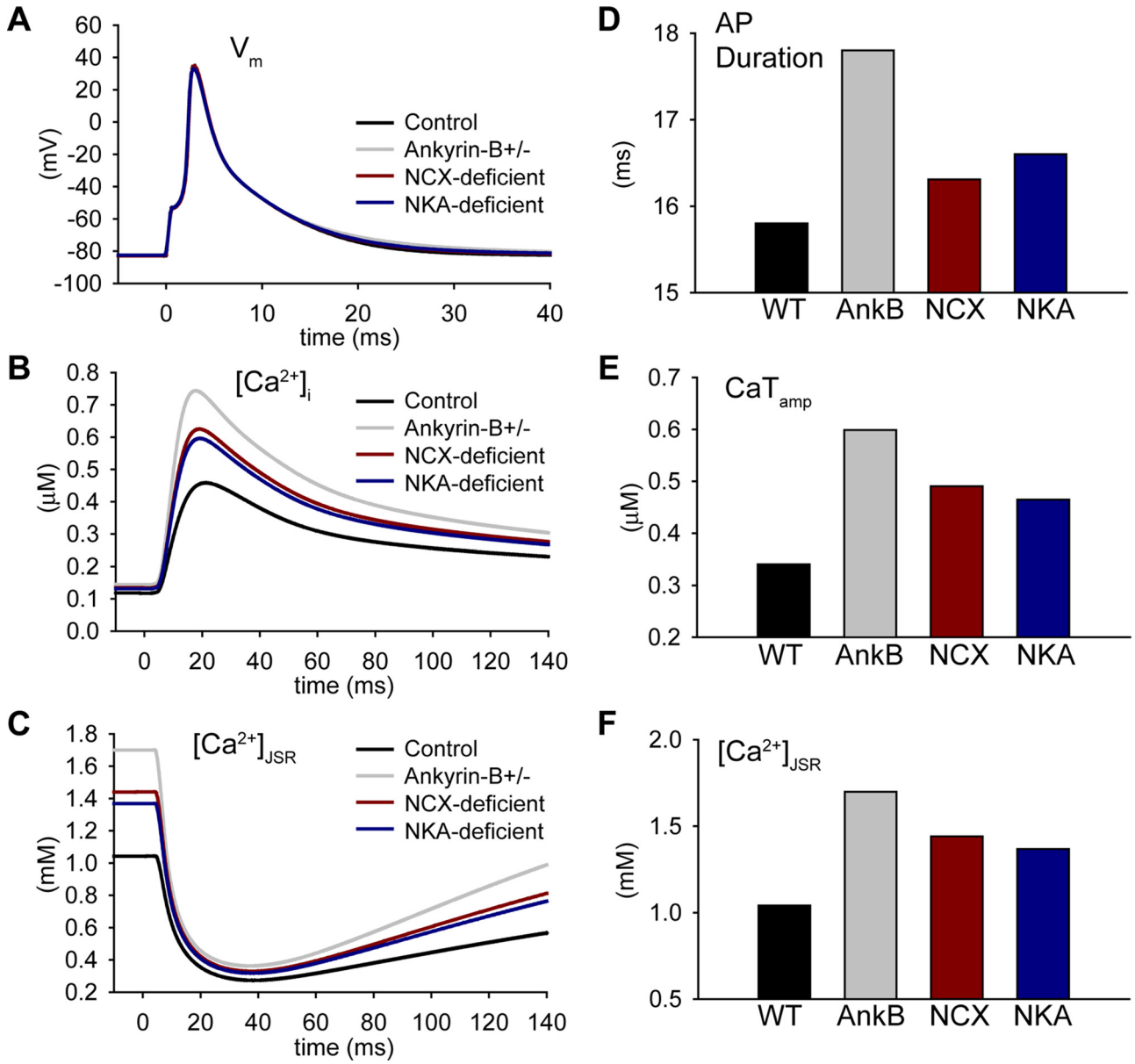


Fig. 4. Role of NCX and NKA in Ca^{2+} accumulation in the ankyrin-$B^{+/-}$ cell. *A–C*: simulated steady-state AP (*A*), Ca^{2+} transient (*B*), and $[Ca^{2+}]_{JSR}$ (*C*) in control, ankyrin-$B^{+/-}$, NCX-deficient (NKA restored to normal levels), and NKA-deficient (NCX restored to normal levels) cells. *D–F*: summary data showing steady-state APD (*D*), CaT_{amp} (*E*), and $[Ca^{2+}]_{JSR}$ (*F*) in control, ankyrin-$B^{+/-}$ (AnkB), NCX-deficient, and NKA-deficient cells (CL = 1,000 ms).

ankyrin-$B^{+/-}$ cell but dramatically reduced Ca^{2+} transient amplitude (Fig. 4, *B* and *E*) and $[Ca^{2+}]_{JSR}$ toward control levels (Fig. 4, *C* and *F*). In contrast, restoring normal NKA targeting in the ankyrin-$B^{+/-}$ cell had a greater impact on APD (Fig. 4, *A* and *D*), with a lesser effect on Ca^{2+} transient amplitude (Fig. 4, *B* and *E*) or $[Ca^{2+}]_{JSR}$ (Fig. 4, *C* and *F*) compared with restoring NCX targeting. NCX loss was also the dominant mechanism responsible for increased Ca^{2+} transient amplitude and $[Ca^{2+}]_{JSR}$ in the human ankyrin-B-deficient cell (Supplemental Fig. S2). Together, these data indicate that NKA and NCX loss play distinct but important roles in ankyrin-B deficient cells. Whereas loss of NKA targeting is primarily responsible for changes in APD in ankyrin-$B^{+/-}$ cells, loss of NCX drives the accumulation of cytosolic and SR Ca^{2+}.

Increased levels of Ca^{2+} with isoproterenol. Human patients with *ANK2* mutations show increased likelihood of stress-induced cardiac arrhythmias (28, 34). Similarly, ankyrin-$B^{+/-}$ mice display lethal ventricular arrhythmias in response to catecholamine injection after exercise (28). Based on these observations, we next determined the response of the ankyrin-$B^{+/-}$ and control models to treatment with the β-adrenergic receptor agonist isoproterenol (Fig. 5). Isoproterenol treatment resulted in a dramatic increase in the Ca^{2+} transient amplitude in both control and ankyrin-$B^{+/-}$ cells (Fig. 5, *B* and *E*) due to increased I_{CaL} (Fig. 5, *C* and *F*) and SR Ca^{2+} uptake (not shown). Spontaneous Ca^{2+} release from the SR did not occur in either the control or ankyrin-$B^{+/-}$ models during slow pacing (CL = 1,000 ms) even in the presence of isoproterenol.

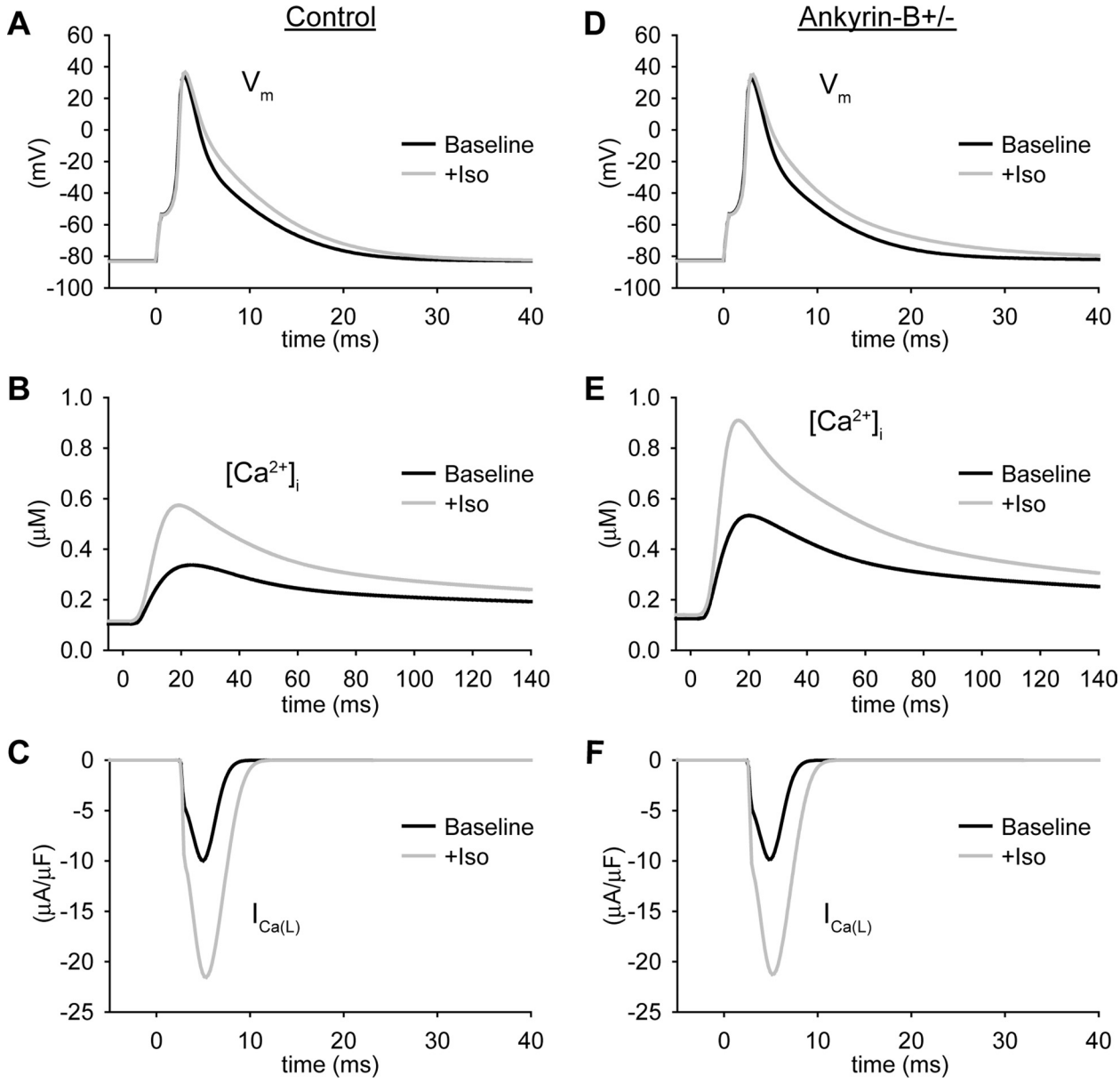
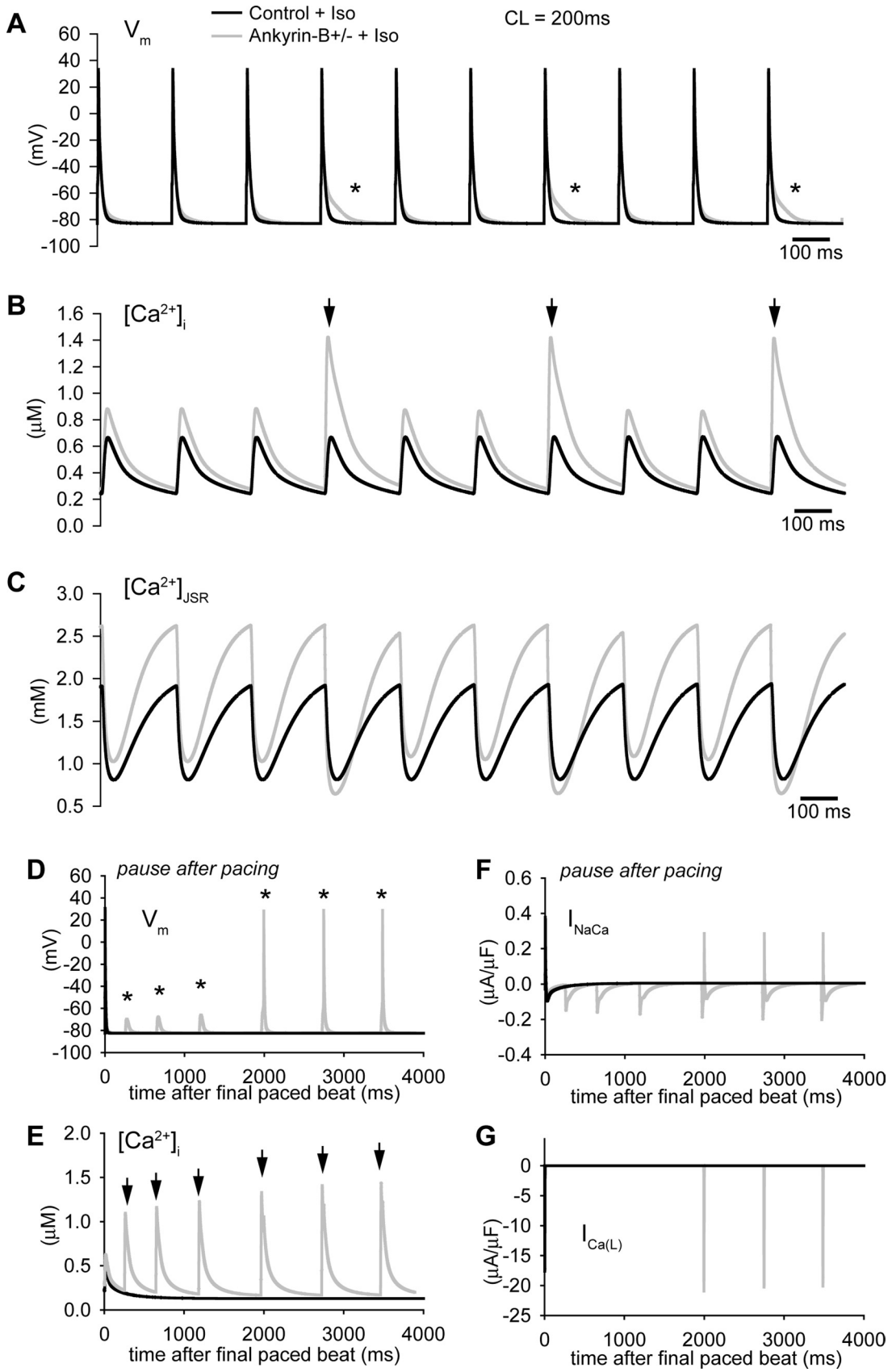


Fig. 5. Isoproterenol (Iso) increases intracellular Ca^{2+} in control and ankyrin-$B^{+/-}$ cells. *A–F*: simulated steady-state AP (*A* and *D*), Ca^{2+} transient (*B* and *E*), and I_{CaL} (*C* and *F*) for control (*A–C*) and ankyrin-$B^{+/-}$ (*D–F*) mouse ventricular cardiomyocytes at baseline and in the presence of a saturating concentration of Iso (> 0.1 μM, CL = 1,000 ms).

We next subjected the control and ankyrin-$B^{+/-}$ models to rapid pacing in the presence of isoproterenol. Interestingly, multiple spontaneous SR Ca^{2+}-release events were observed in the ankyrin-$B^{+/-}$ model during rapid pacing (CL = 200 ms; arrows in Fig. 6*B*) due to the increased SR Ca^{2+} load (Fig. 6*C*). These spontaneous release events altered AP morphology during pacing (asterisks in Fig. 6*A*) and even produced afterdepolarizations that successfully triggered APs during a subsequent pause (Fig. 6*D*). Spontaneous release and afterdepolarizations did not occur in the control model during rapid pacing (Fig. 6, *A* and *B*) or a subsequent pause (Fig. 6, *D* and *E*) in the presence of isoproterenol. These simulations indicate that ankyrin-B reduction increases the likelihood of spontaneous release and AP afterdepolarizations. Consistent with experimental observations, afterdepolarizations were only observed during rapid pacing rates (such as exercise) and in

Fig. 6. Spontaneous Ca^{2+} release and afterdepolarizations in the ankyrin-$B^{+/-}$ cell during rapid pacing in the presence of Iso. *A–C*: simulated AP (*A*), Ca^{2+} transient (*B*), and $[Ca^{2+}]_{JSR}$ (*C*) in control and ankyrin-$B^{+/-}$ mouse ventricular cardiomyocytes during rapid pacing to steady state (CL = 200 ms) in the presence of Iso. Frequent spontaneous release events (arrows in *B*) led to abnormal repolarization (* in *A*) in the ankyrin-B-deficient cell. *D–G*: simulated AP (*D*), Ca^{2+} transient (*E*), I_{NaCa} (*F*), and I_{CaL} (*F*) in control and ankyrin-$B^{+/-}$ cells during a subsequent pause after rapid pacing to steady state. Note the spontaneous release (arrows) and afterdepolarizations (*) that ultimately produced an AP.



the presence of isoproterenol (β-adrenergic stimulation) (28). We observed similar behavior in simulations with the human model (Supplemental Fig. S3). Specifically, spontaneous release was observed during rapid pacing in the presence of isoproterenol in the ankyrin-B$^{+/-}$ cell but not in the control cell (Supplemental Fig. S3*A*). In fact, spontaneous release produced afterdepolarizations that generated APs during pacing in the human model (asterisk in Supplemental Fig. S3*A*).

To determine the relative contributions of NCX and NKA loss to Ca^{2+} overload, spontaneous release, and afterdepolarizations, we subjected the ankyrin-B$^{+/-}$ cell with normal NCX targeting or with normal NKA targeting to rapid pacing in the presence of isoproterenol (Fig. 7). Restoring NCX targeting greatly reduced the occurrence of spontaneous release and afterdepolarizations in ankyrin-B$^{+/-}$ cells (number of events decreased >5-fold in NKA-deficient compared with ankyrin-B$^{+/-}$ cells; Fig. 7, *A*, *B*, and *D*). While restoring normal NKA targeting had a lesser impact on spontaneous release, it reduced the number of events and delayed the time to first spontaneous release (compare ankyrin-B- and NCX-deficient cells in Fig. 7, *C* and *D*). Consistent with these findings, loss of NCX resulted in more frequent and earlier spontaneous release events than NKA loss in the human model (Supplemental Fig. S3, *B* and *C*). These data indicate that while both NCX and NKA loss contribute to SR Ca^{2+} overload and afterdepolarizations in ankyrin-B deficient cells, loss of NCX is the dominant mechanism.

DISCUSSION

NKA and NCX are functionally coupled transporters for the regulation of ion homeostasis and contractility in the heart (10, 31, 37). Specifically, NKA maintains the Na^+ gradient across the membrane necessary for NCX to remove Ca^{2+} from the cell. For centuries, this coupling has been exploited for the treatment of heart failure symptoms through the use of digitalis (18). However, only in the past 50 yr has the direct link between digitalis, NKA, and contractility been established. Recent studies (23, 30) have revealed that functional coupling of NKA and NCX in fact depends on physical coupling of the transporters. In fact, ankyrin-B binds to both NKA and NCX and is required for normal membrane expression of NKA and NCX (23). Loss of ankyrin-B disrupts targeting of NCX and NKA as well as $InsP_3$ receptor and PP2A and is linked to arrhythmia in humans and mice (2, 7, 23–25, 28–30). Our data indicate that loss of NCX and NKA targeting in ankyrin-B$^{+/-}$ cells promotes intracellular Ca^{2+} accumulation at baseline and spontaneous Ca^{2+} release and afterdepolarizations with rapid pacing in the presence of isoproterenol. These findings agree with experimental observations in both ankyrin-B$^{+/-}$ mice and human patients (26, 28, 29). Whereas NKA mistargeting is primarily responsible for AP prolongation in ankyrin-B$^{+/-}$ cells, loss of NCX is the dominant mechanism for intracellular Ca^{2+} accumulation, spontaneous release, and afterdepolarizations. In light of these findings, it is surprising that, in contrast to ankyrin-B$^{+/-}$ mice, NCX1 knockout mice have normal

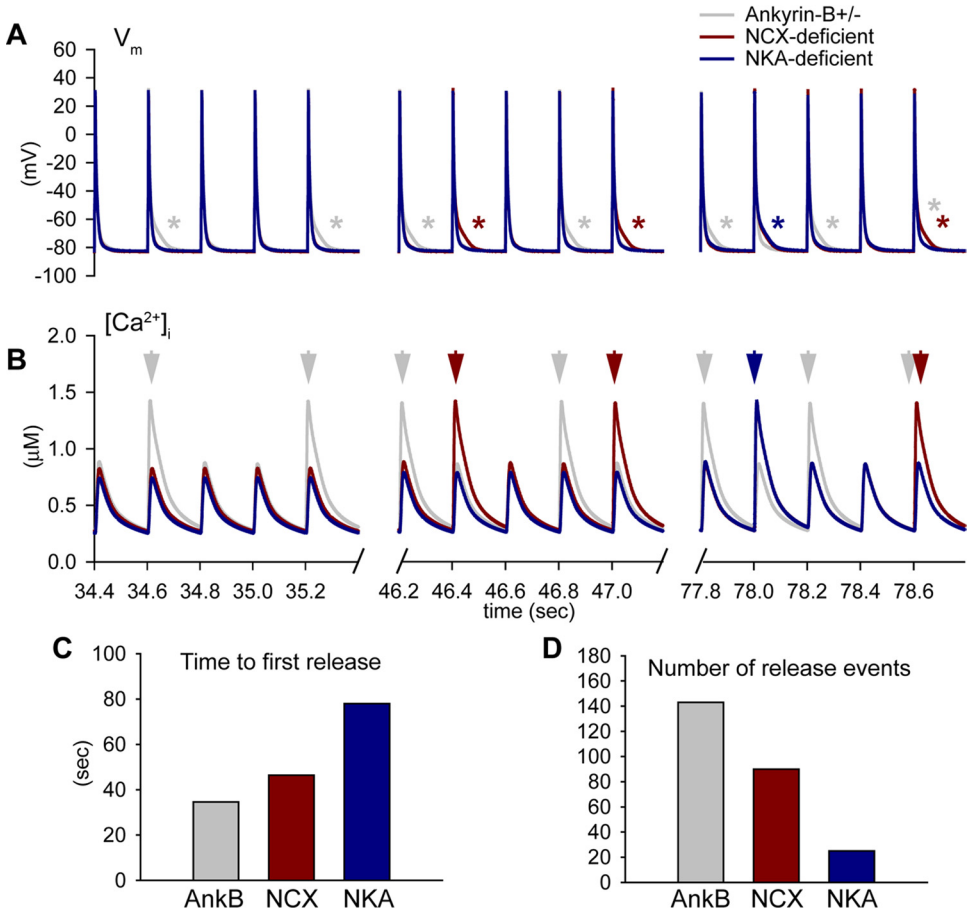


Fig. 7. Role of NCX and NKA in spontaneous Ca^{2+} release and afterdepolarizations in the ankyrin-B$^{+/-}$ cell. *A* and *B*: simulated AP (*A*) and Ca^{2+} transient (*B*) in ankyrin-B$^{+/-}$, NCX-deficient, and NKA-deficient cells during rapid pacing to steady state (CL = 200 ms). Spontaneous Ca^{2+} release (arrows in *B*) and abnormal repolarization (asterisks in *A*) were observed in NCX-deficient and NKA-deficient cells, although with decreased frequancy and delayed onset compared with the ankyrin-B$^{+/-}$ cell. *C* and *D*: summary data showing the time to first spontaneous release of Ca^{2+} from the SR (*C*) and number of spontaneous release events during rapid pacing (*D*) in ankyrin-B$^{+/-}$, NKA-deficient, and NCX-deficient cells.

Ca^{2+} transients with a modest decrease in contractility and normal lifespan (13). However, unlike ankyrin-B-deficient mice, NCX1 knockout mice show a compensatory decrease in I_{CaL}, which limits Ca^{2+} influx and likely mitigates the detrimental effects of NCX loss (13). Future studies are required to determine the mechanism for I_{CaL} downregulation in NCX1 mice and the reason for differential regulation in NCX1 knockout and ankyrin-B-deficient mice.

It is important to note that additional factors not accounted for in the model may regulate triggered activity in ankyrin-B deficiency. One possibility includes an altered phosphorylation state of SR and/or membrane target proteins due to loss of PP2A activity (2). In fact, reduced expression of the B56α regulatory subunit of PP2A has been shown to cause Ca^{2+}/calmodulin-dependent kinase II-dependent hyperphosphorylation of ryanodine receptor 2 and afterdepolarizations (39). Moreover, Ca^{2+} overload itself due to loss of NKA and NCX may result in abnormal kinase activity (33). Thus, perturbation of kinase/phosphatase regulation in ankyrin-B deficiency has the potential to exacerbate dysfunction due to loss of NKA and NCX targeting.

While not a consistent clinical finding, a subpopulation of patients with ankyrin-B syndrome present with prolongation of the QT interval on the electrocardiogram (26, 28, 34). The mechanism responsible for this phenotype is unclear. Our simulation results indicate that loss of NKA is responsible for the slight prolongation of APD in ankyrin-$B^{+/-}$ cells. Nonetheless, it is unclear whether QT prolongation is due to AP prolongation or another mechanism [e.g., abnormal conduction (28)]. Future studies are required to address QT prolongation in the subpopulation of ankyrin-B syndrome patients with long QT.

A recent study (11) from our group has identified Eps15 homology domain (EHD) proteins as a new class of cardiac proteins involved in ankyrin-B-based targeting of NCX. EHD knockdown in wild-type neonatal cardiomyocytes reduces NCX membrane expression and function. Conversely, EHD overexpression increases cell surface NCX and current. The results from the present study support the idea that restoring NCX function by targeting ankyrin and/or EHD may be an effective strategy for preventing cardiac arrhythmia and sudden death in patients with congenital or acquired ankyrin-B deficiency.

Limitations. Our model of the ankyrin-$B^{+/-}$ cardiomyocyte is based closely on cellular data from an animal model of ankyrin-$B^{+/-}$ that phenocopies human patients with ankyrin-B syndrome. While this effort is an important first step, it is important to note that our model is limited by the available experimental data. While loss of current and surface expression of NCX and NKA are well documented in ankyrin-$B^{+/-}$ mice, data on NCX and NKA biophysical properties are limited. However, it has been shown that while there is a loss of ouabain-binding sites (parallels loss of NKA) in ankyrin-B-deficient cardiomyocytes, the affinity for ouabain is unchanged for residual sites (25). These data, while far from conclusive, suggest that the population of NKA that targets successfully in the absence of ankyrin-B functions normally. Furthermore, while the model addresses loss of NCX and NKA targeting in ankyrin-B-deficient cells, it does not account for defects in the localization of the $InsP_3$ receptor (or PP2A, as discussed above) in ventricular cardiomyocytes. In fact, the role of the $InsP_3$ receptor in cardiomyocytes is unclear, particularly in the ventricle, where Ca^{2+} release is controlled by ryanodine receptor Ca^{2+}-release channels. We expect our model to serve as a quantitative framework into which new data on $InsP_3$ function (and PP2A) in ventricular cardiomyocytes may be incorporated to determine the consequences of $InsP_3$ mislocalization in ankyrin-$B^{+/-}$ cells.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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