Sex-differences in sodium/calcium exchange expression is a determinant of the arrhythmia phenotype in pre-pubertal rabbit hearts with Long QT type 2

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Abstract

Background—Adult females are more prone to Torsade de Pointes (TdP) than males in long QT type 2 (LQT2) elicited by inhibiting the K⁺ current, I_{Kr}. Sex-differences in the propensity to early afterdepolarizations (EADs) and TdP are reversed in adolescence and correlate with higher levels of L-type Ca²⁺ channels and current, I_{Ca,L} at the base of the epicardium in adolescent male rabbit hearts, even though female hearts have longer action potential (AP) durations. Higher Ca²⁺-influx via I_{Ca,L} should be balanced by higher Ca²⁺-efflux.

Objectives—Sex-differences in Na⁺-Ca²⁺ exchanger and its current, I_{NCX} are investigated in adolescent rabbit hearts to determine if they parallel sex-differences in I_{Ca,L}.

Methods and Results—Optical APs and intracellular Ca²⁺ (Ca_i) were mapped in pre-pubertal Langendorff rabbit hearts. E4031 (0.5μM) blocked I_{Kr}, elicited EADs and TdP in male (n=10) but not female (n=18) hearts. KB-R4379 (I_{NCX} inhibitor, 1μM) reversed (n=5/5) or protected from EADs if perfused before E4031 (n=6/6). I_{NCX} density was 20–30% greater in base compared to apex myocytes of pre-pubertal male (p<0.02) or to apex and base myocytes of female hearts (p<0.02). In Western blots, NCX was 22% higher at the base of male versus female hearts and 25% higher than the apex (n=4, each gender). Immuno-histochemistry of ventricular sections revealed higher NCX levels in male versus female hearts.

Conclusions—Sex and regional differences in NCX correlate with L-type Ca²⁺ channels and NCX inhibition suppresses EADs and TdP in LQT2. These data provide compelling evidence that sex-differences in Ca²⁺ handling determine the arrhythmia phenotype.

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Keywords

Sex-differences in Arrhythmia vulnerability; Pre-pubertal versus adult; Sodium/calcium exchange blocker KB-R4379; I_{K_r} blocker, E4031; Optical mapping

Introduction

Mutations of *KCNH2*, the gene that encodes for the channel protein HERG, responsible for the delayed rectifying K^+ current, I_{K_r} have been linked to the phenotype associated with Long QT type 2 (LQT2).¹ In congenital and drug-induced forms of LQT2, the I_{K_r} loss-of-function prolongs the cardiac action potential duration (APD), the QT interval, the propensity to early afterdepolarizations (EADs) and Torsade de Pointes (TdP), a form of polymorphic ventricular tachycardia.² Ca^{2+} overload and spontaneous rise of intracellular free Ca^{2+} (Ca_i) have been implicated in LQT2 as mechanisms that trigger EADs and TdP.³ APD prolongation increases Ca^{2+} -entry via L-type Ca^{2+} -channels causing an enhanced accumulation of Ca^{2+} in the sarcoplasmic reticulum (SR), spontaneous SR Ca^{2+} release, a local elevation of Ca_i near T-tubules, which activates depolarizing Na-Ca exchange current, I_{NCX} that in turn re-activates $I_{Ca,L}$.^{3–7} Studies on isolated myocytes and various heart preparations have implicated SR Ca^{2+} release as the mechanism for the generation of EADs.^{4, 8, 9} Simultaneous optical mapping of action potentials (APs) and Ca_i transients showed that Ca_i elevation preceded the firing of EADs induced by E4031. EADs appeared at specific foci (typically 2–3 sites when imaging the anterior surface of the rabbit heart), captured and propagated across the epicardium until collisions annihilated the wave fronts.¹¹ Phase maps showed that during a normal cardiac beat voltage preceded Ca_i resulting in a counter-clockwise trajectory. In contrast, at the site of an EAD, the trajectory became clockwise (i.e. Ca_i preceded the voltage depolarization) and the magnitude and kinetics of Ca_i indicated that spontaneous SR Ca^{2+} release was the most likely source of Ca^{2+} -triggered EADs.¹¹

An interesting aspect of LQT2 is the reversal of TdP risk in pre-pubertal rabbits, before the surge of sex hormones (in 42-day old rabbits)^{12, 13} where male hearts are more likely to have E4031-induced TdP than female hearts even though females have longer APDs.¹⁴ The reversal of TdP-risk in pre-pubertal rabbits is similar in humans where boys (< 14 years old) with inherited LQT2 are more likely to fall victim of lethal arrhythmias than girls.¹⁵ The shorter APDs in adolescent male rabbit hearts, yet their greater likelihood of EADs and TdP than in pre-pubertal females indicates that i) APD prolongation alone does not predict the arrhythmia phenotype, ii) the surge of sex-steroids and reversal of arrhythmia risk in adults vs. adolescents implicates a genomic regulation of ion channel expression by sex steroids, and iii) sex-differences in Ca_i handling and Ca^{2+} overload in LQT2 may underlie sex-differences in arrhythmia phenotype in LQT2. Optical mapping of APs and Ca_i transients showed that EADs (in adult female and pre-pubertal male hearts) preferentially started at the base of ventricles which coincided with regions with a greater density of L-type Ca^{2+} channel protein, Cav1.2, mRNA levels and current, $I_{Ca,L}$ compared to the apex and to hearts from the opposite sex.¹⁶ In Langendorff hearts and isolated myocytes, higher $I_{Ca,L}$ density was shown to be associated with greater Ca^{2+} influx which in the setting of prolonged APDs caused SR Ca^{2+} overload and increased the incidence of EADs.¹⁶ Moreover, myocytes isolated from the base of adult female and pre-pubertal male hearts had marked EADs when treated with E4031 whereas myocytes from the opposite sex, adult male and pre-pubertal female myocytes were protected from E4031-induced EADs.¹⁶

In the present study, we investigated differences in NCX expression in pre-pubertal rabbits that might parallel our findings of sex, age and regional differences of L-type Ca^{2+} channel expression.¹⁶

MATERIALS AND METHODS

Heart preparations

This investigation conformed to the Institutional Animal Care and Use Committee of the University of Pittsburgh and the current *Guide for Care and Use of Laboratory Animals* published by the National Institutes of Health. New Zealand white rabbits (6-weeks old; 1.6 kg) were injected with pentobarbital (50 mg kg^{-1} , iv) plus heparin (200 U kg^{-1}), then the heart was excised and perfused with Tyrode's solution containing (in mM): 130 NaCl, 24 NaHCO_3 , 1.0 MgCl_2 , 4.0 KCl, 1.2 NaH_2PO_4 , 50 Dextrose, 1.0 CaCl_2 , at pH 7.4, gassed with 95% O_2 and 5% CO_2 . The atrioventricular node was crushed with forceps to pace and control heart rate. Hearts were stained with a voltage-sensitive dye (PGH1, 10 μl of 1 mg/ml of dimethyl sulfoxide, DMSO)¹⁷ and a Ca^{2+} indicator (Rhod-2/AM, 0.3 ml of 1 mg/ml DMSO). EKGs and perfusion pressure were continuously monitored from hearts placed in a chamber to reduce movement artifacts, without the use of electromechanical uncouplers. Temperature was maintained at $37.0 \pm 0.2^\circ\text{C}$ and perfusion pressure was adjusted to $\sim 70 \text{ mmHg}$.¹¹ Long QT, EADs and TdP were induced by adding the I_{Kr} blocker, E4031 ((1-[2-(6-methyl-2-pyridyl)-ethyl]-4-(4-methylsulfonylaminobenzoyl) piperidine = $0.5 \mu\text{M}$). More details are available in the "SUPPLEMENT TO METHODS".

Data Analysis

I_{NCX} density was compared between pre-pubertal male and female myocytes isolated from the base and apex of the hearts for n: number of cells and N: number of hearts. Student t-test was used to compare values at +60 mV with statistical significance for $p < 0.05$ for male, base vs. apex, male base vs. female base and for female base vs. apex. To better evaluate sex-differences of I_{NCX} on the cardiac action potential, the analysis was performed for physiological ranges of inward (-80 to -20 mV) and outward (0 to $+20 \text{ mV}$) I_{NCX} currents, as shown in Table 1. Data distributions were examined using Shapiro-Wilk W-test for normality. Since most distributions were significantly different from normal, nonparametric tests were applied, unless otherwise indicated in the text. The apex versus base data in the same hearts were compared using Wilcoxon matched pairs test. The male versus female data were compared using Mann Whitney U test.

Results

Sex Differences in LQT2 Arrhythmia Phenotype

Sex was shown to be a strong determinant of the arrhythmia phenotype in pre-pubertal rabbits with drug-induced LQT2. As illustrated in Figure 1 (in part from ref 14 and 16), the I_{Kr} blocker, E4031 ($0.5 \mu\text{M}$) prolonged AP durations, elicited EADs that progressed to TdP in pre-pubertal male hearts within minutes ($n = 7$ out of 10 hearts). Calibrations of Rhod-2 Ca_i signals estimated the diastolic and systolic Ca_i to be 300 and 800 nM, respectively. E4031 changed the time course but not the range of diastolic and systolic Ca_i .¹¹ E4031 prolonged APDs but failed to elicit EADs or TdP in pre-pubertal female rabbit hearts for over 30 min (16 out of 18). In 2 out of 18 pre-pubertal female hearts, E4031 elicited brief arrhythmias that terminated spontaneously (male vs. female, $p < 0.01$).¹⁴

Sodium/Calcium Exchanger as a Determinant of Arrhythmia Phenotype

The role of Ca^{2+} elevation as a trigger of EADs in pre-pubertal female hearts was evaluated by perfusing with E4031 ($0.5 \mu\text{M}$) then raising extracellular Ca^{2+} from 1 to 1.8 mM. Higher

Ca^{2+} elicited brief bursts of depolarization during the plateau phase which spontaneously subsided (5–10 s) (Figure 2) (n=4 out of 4 hearts). The NCX inhibitor, KB-R7943 was also tested as a method to suppress EADs and TdP. Pre-pubertal hearts did not exhibit significant changes in amplitude and kinetics of APDs and Ca_i transients before (Figure 2C) and after perfusion with KB-R7943 (1 μM) only for 10 minutes (n=4 hearts) (Figure 2D) and with a range of cycle lengths of 400 to 1000 ms for over 30 min (n=2). Pre-pubertal male hearts had the expected salvos of EADs upon perfusion with E4031 (Figure 3B). The co-perfusion with KB-R7943 (1 μM) suppressed EADs after 30 min but not within 10 min (panel C vs. D) and suppressed TdP in 6 out of 6 hearts (Fig. 3C). The inhibition of EADs by KB-R7943 lasted for the duration of the experiment (>45 min).

We previously reported that I_{Kr} inhibition elicited EADs in myocytes isolated from the base-epicardium of pre-pubertal male but not pre-pubertal female hearts.¹⁶ Figure 4 shows that the suppression of I_{Kr} with dofetilide elicits EADs in pre-pubertal male base (panel B, n= 5 out of 5) but not apex (panel A, n= 0 out of 5) myocytes. Moreover, the subsequent addition of KB-R7943 (1 μM) suppressed EADs at the single cell level during the co-perfusion with dofetilide (200 nM) (Figure 4B).

Sex and regional Heterogeneities of NCX

Western blot analysis of membrane fractions isolated from pre-pubertal male and female myocardium revealed a higher level (~ 2-fold) of NCX1 at the base of male compared to female left ventricles (N=3 hearts, each sex) (Figure 5A). Here, the apparent molecular weight of NCX1 was ~ 70 kDa which is the expected cleavage product of NCX1 (M_r ~ 120 kDa). In these experiments, the risk of LQT2-related arrhythmias was first studied by optical mapping then tissues were prepared for Western blots. TdP was induced in the three pre-pubertal male but not the three female hearts. In another set of experiments, protein samples were freshly prepared from the base and apex of the epicardium of pre-pubertal male and female hearts (n=4 each). As shown in Figure 5B & C, NCX1 had the correct molecular weight (M_r ~ 120 kDa) and was markedly higher at the base of four pre-pubertal male hearts compared to the base of pre-pubertal female hearts (panel B) and compared to the apex of pre-pubertal male and female hearts (panel C). Protein densities (normalized with respect to -actin) showed that NCX1 was higher at the base of pre-pubertal male hearts.

The NCX1 tissue density was measured by immuno-histochemistry by sectioning whole hearts to visualize NCX1 distribution. NCX1 density was visibly greater (n=3; $p<0.05$) in adolescent male than female hearts (Figure 6). The NCX current (I_{NCX} density (pA/pF) was measured from myocytes isolated from the base and apex of adolescent female and male hearts and the current-to-voltage relationship was plotted for each group of myocytes (Figure 7A). As summarized in Figure 7B, I_{NCX} density measured at +60 mV was greater at the base than apex of male myocytes (n= 7 cells N=3 hearts, $p<0.05$) and male base I_{NCX} was statistically greater compared to female base and apex myocytes. In pre-pubertal female myocytes, there was no significant difference in I_{NCX} at the base and apex (NS, $p=0.58$). Data were analyzed for their physiological significance by measuring the mean $\pm$ SD of the inward and outward I_{NCX} in the voltage range that span the cardiac action potential, respectively -80 to -20 mV and from 0 to +20 mV. As shown in Table 1, the mean *inward* I_{NCX} density at the base of male myocytes was 49% greater than at the apex ($p<0.001$) and was 100% greater than female base ($p=0.014$) and 64% greater than female apex ($p<0.01$) myocytes. The mean *outward* I_{NCX} density at the base of male myocytes was 61% greater than at the apex ($p<0.05$) and was 3-fold greater than female base ($p=0.0005$) and apex ($p<0.001$) myocytes. It is reasonable to expect that these significant sex-differences in I_{NCX} density in both the forward (*inward* I_{NCX}) and reverse (*outward* I_{NCX}) modes of the exchanger during an action potential will have a considerable influence on Ca^{2+} influx and efflux.

Discussion

The main findings are that sex differences in arrhythmia phenotype in LQT 2 are reversed in pre-puberty compared to adulthood and that long QT or long APDs alone cannot be used as a predictor of arrhythmia vulnerability. In pre-pubertal female hearts, perfusion with the I_{K_r} blocker E4031 prolonged APDs from 200–300 ms to 1–2 s, yet did not elicit EADs or TdP whereas in male hearts E4031 triggered salvos of EADs and TdP within minutes. Besides APD prolongation, intracellular Ca^{2+} handling is an important determinant of arrhythmia vulnerability since spontaneous elevation of Ca^{2+} in the cytoplasm was shown to precede EADs.¹¹ In drug induced LQT 2, NCX may underlie sex differences in arrhythmia phenotype since a) pharmacological inhibition of I_{NCX} interrupted EADs and TdP in pre-pubertal male hearts with LQT2, b) Western blot analysis indicated a 22% greater protein levels in male compared to female hearts, c) I_{NCX} density is greater at the base of the pre-pubertal male epicardium compared to I_{NCX} at the base and apex of pre-pubertal female epicardium, and d) in pre-pubertal male hearts, EADs fired first at the base of the epicardium where I_{NCX} and $I_{Ca,L}$ are elevated.¹⁶ The data imply that sex-differences in NCX expression result in marked differences in Ca_i handling and arrhythmia phenotype in LQT2.

Pharmacology of KB-R7943

NCX inhibition has been considered as a potential target to treat heart failure and reperfusion arrhythmias.^{18, 19} In ischemia, anaerobic metabolism leads to intracellular acidosis, increases in intracellular $[Na^+]$ which affects NCX by decreasing the driving force for Ca^{2+} efflux. KB-R7943 was first reported to be an inhibitor of the reverse mode (1 Ca^{2+} in vs. 3 Na^+ out)¹⁹ but later studies reported an effective inhibition of the forward mode (3 Na^+ in vs. 1 Ca^{2+} out). KB-R7943 inhibits NCX from the external side with relatively fast kinetics and its action can be reversed by washing the drug.¹⁸ KB-R7943 inhibited NCX with variable potency in different cardiac myocytes; in rat myocytes IC_{50} was 1.2 to 2.4 μM for the reverse mode and > 30 μM for the forward mode. In guinea pig myocytes, IC_{50} s were lower with values of 0.32 and 17 μM for the reverse and forward modes respectively.²⁰ The selectivity of KB-R7943 for NCX has been challenged. In guinea pig myocytes, the drug was reported to inhibit I_{Na^+} , $I_{Ca,L}$ and I_{K1} with IC_{50} s of 14, 8 and 7 μM , respectively.²⁰ Due to the potential of poor selectivity of the drug in intact hearts, the current study did not test the effects of KB-R7943 >1 μM to avoid difficulties in the interpretation of the results. The suppression of EADs and TdP by 1 μM KB-R7943 suggests that inhibition of the reverse mode of NCX may be sufficient to abate EADs because reverse NCX could be a major contributor of Ca^{2+} influx and Ca_i overload in LQT2. However, controversies persist regarding the magnitude and significance of reverse NCX, as a component of Ca^{2+} entry and as a trigger of SR Ca^{2+} release.²¹ In LQT2, the depolarizing inward NCX current is thought to provide the trigger to reactivate $I_{Ca,L}$ during the plateau phase of the AP and thereby cause EAD upstrokes. Higher NCX expression could increase Ca_i load by reverse NCX or decrease Ca_i load by forward NCX and would be expected to increase the magnitude of the current needed to trigger EADs. Future studies will investigate the relative potency of agents that inhibit the forward vs. reverse modes of NCX on the suppression of EADs and TdP.

NCX and Arrhythmia Vulnerability

Pharmacological intervention of reverse mode NCX and changes in NCX expression have been used to evaluate the contribution of I_{NCX} to arrhythmias. Perfusion of KB-R7943 (3 μM) for 10 min prior to global ischemia (30 min) protected rabbit hearts from tachyarrhythmia and fibrillation.²² The over-expression and under-expression of NCX in molecularly engineered mice respectively enhanced and reduced the susceptibility to reperfusion arrhythmias.^{23, 24} In a rabbit model of heart failure, NCX elevation increased

I_{NCX} which explained the reduction of Ca_i transients, force and increased the propensity for triggered arrhythmias by increasing delayed afterdepolarizations (DADs).²⁵ In contrast, the inhibition of NCX by KB-R7942 was reported to enhance arrhythmogenicity by increasing the incidence of APD alternans in canine hearts during rapid pacing.²⁶ There is little doubt that NCX plays an important role in excitation-contraction coupling and in the maintenance of the normal contraction cycle.²⁷ Thus far, the literature demonstrates an important role for NCX in arrhythmia vulnerability but did not consider sex differences in NCX expression. Perhaps the more important parameter to examine is the balance of NCX and other Ca^{2+} channels and transporters which are needed to define the substrate and the vulnerability to arrhythmias.

Sex Differences in NCX expression

The present report identifies NCX as an important component of sex differences and arrhythmia phenotype in pre-pubertal rabbit hearts. Western blot protein analysis, I_{NCX} density and immuno-histochemistry from male vs. female hearts support the notion that NCX expression is higher in pre-pubertal male than female hearts in a region-dependent manner and this heterogeneity is reversed in adulthood. Furthermore, the arrhythmia phenotype in LQT2 is also reversed post-puberty and is linked to higher levels of NCX and L-type Ca^{2+} channel expression. The main function of NCX is to extrude Ca^{2+} and on a beat-to-beat basis, reverse Ca^{2+} entry via L-type voltage gated Ca^{2+} channels. Thus, Ca^{2+} influx via $I_{Ca,L}$ is balanced by Ca^{2+} efflux via I_{NCX} and Ca^{2+} pumps on the plasma membrane. The balance of these currents is critical to reset intracellular Ca^{2+} since any imbalance will lead to a gradual but inevitable depletion or over-accumulation of Ca^{2+} in heart cells.

For these reasons, we examined the possibility that sex-differences in $I_{Ca,L}$ reflect changes in I_{NCX} seen in adult and pre-pubertal rabbit hearts. Our recent findings are that $I_{Ca,L}$ and Ca^{2+} channel protein, Cav1.2 were elevated whenever I_{NCX} was elevated at the base of the heart. This means that $I_{Ca,L}$ and Cav1.2 are higher at the base of adult female rabbit hearts compared to the apex of the same hearts and to the base and apex of adult male rabbit hearts. Conversely, $I_{Ca,L}$ and Cav1.2 are higher at the base of pre-pubertal male rabbit hearts compared to the base and the base and apex of pre-pubertal female hearts. These findings imply that in drug-induced long QT or conditions that prolong APDs, adult female and pre-pubertal male hearts are more likely to exhibit intracellular Ca^{2+} overload compared to their adult male and pre-pubertal female counterpart due to the higher expression of Ca^{2+} handling channels. Ca^{2+} overload in the lumen of the SR will increase the propensity to spontaneous SR Ca^{2+} release, elicit a depolarizing I_{NCX} which can re-activate L-type Ca^{2+} channels and thereby elicit EADs.^{11, 14} The incidence of EADs is a singular risk factor to the initiation of arrhythmias like TdP. Put together, these findings implicate NCX as an important determinant of sex differences in electrophysiological properties and of arrhythmia phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

APD	action potential duration
V_m	membrane potential
Ca_i	[Ca ²⁺] _i , intracellular free Ca ²⁺ concentration
EADs	early afterdepolarizations
TdP	Torsade de Pointes
LQT2	Long QT type 2
I_{Kr}	rapid component of the delayed rectifying K ⁺ current
NCX	sodium/calcium exchanger and its current, I _{NCX}

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A. CONTROL

B. + E4031

Figure 1. Model of LQT2 in Pre-pubertal male rabbit hearts

A: Control optical AP from the center of the epicardium on a pre-pubertal male heart.
 B: Perfusion with E4031 (0.5 μ M) caused an APD prolongation (a) followed by EADs (b) in ~ 5 min. EADs frequency increased (c) and progressed to TdP within 10 min. In pre-pubertal female hearts, E4021 increased APDs without eliciting EADs or TdP.¹⁴

Figure 2. Effect of high $[Ca^{2+}]_o$ and KB-R7943 on AP and Cai transient

A: AP and Ca_i transient measured from the base of a pre-pubertal female epicardium after perfusion with E4031 (0.5 μM). Note the absence of EADs and the irregular beat due to prolonged APD and refractory periods.

B. After raising $[Ca^{2+}]_o$ from 1.0 to 1.8 mM in the perfusate, salvos of EADs appeared then subsided without progressing to TdP. High Ca^{2+} did not promote E4031-induced TdP in pre-pubertal female hearts.

C. Control AP, Ca_i transient and a bipolar electrogram (BE)

D. Perfusion with KB-R7943 for 10 min had no statistically significant effect on APD and Ca_i transients.

Figure 3. Effect of E4031 and KB R7943 on Pre-Pubertal Male Heart

A: Control AP, Ca_i transient and bipolar electrogram (BE) from the center of the epicardium
 B: Perfusion with E4031 initiated salvos of EADs that normally increased in incidence but the addition of KBR7943 suppressed EADs (C) and 30 min later prevented the progression to TdP, even in the presence of E4031 in the perfusate.

Figure 4. KB-R7943 suppresses EADs in pre-pubertal male base myocytes

Ventricular myocytes were isolated from the base and apex of pre-pubertal male epicardium to measure action potentials under current clamp conditions. E4031 (200 nM) prolonged APDs in apex myocytes without eliciting EADs (panel A) (n=0 out of 5 myocytes, 2 hearts). In myocytes from the base, E4031 prolonged the APD and elicited prominent EADs (*) which were suppressed by 1 μ M KB-R7943 (n= 5 out 5 myocytes, 2 hearts).

Figure 5. Westerns of NCX density from the base of Pre-Pubertal Hearts

Hearts were mapped optically before preparation of tissues for Western blots. A: NCX1 levels from 3 female (F1, F2 and F3) and 3 male (M1, M2 and M3) hearts, 40 μ l/lane (top). A 50 kDa band from a Coomassie Blue gel was used as a reference (40 μ l/lane) (bottom lanes) NCX was statistically higher in pre-pubertal male compared to female hearts ($n = 3$ hearts for each sex, $p < 0.01$).

In freshly prepared tissue samples from four pre-pubertal male (PM1-PM4) and four pre-pubertal female (PF1-PF4) hearts NCX1 densities were higher at the base of pre-pubertal male and female hearts (B) and were higher than NCX1 at the apex of the same hearts PM1 to PM4 and PF1 to PF4 (C). D: NCX1 density normalized with respect to β -actin was 22% higher at the base of pre-pubertal male than in female hearts ($n=4$, $p<0.05$) and was 25% higher than the apex of both sexes ($n=4$, $p<0.05$).

Pre-pubertal Male Left Ventricular Section anti NCX Ab

Pre-pubertal Female Left Ventricular Section anti NCX Ab

Figure 6. NCX distributions in pre-pubertal male and female left ventricles
Immunohistochemistry revealed a higher expression of NCX in pre-pubertal males than females (n=3 pairs) along the epicardium as well as across the ventricular wall.

Figure 7. I_{NCX} densities at the base and apex of pre-pubertal male and female hearts

A: Current-to-voltage (I-V) plots of I_{NCX} , the Ni-sensitive current (pA /pF) were averaged for myocytes isolated from the base (n/N=7/3) and apex (n/N=6/3) of pre-pubertal male and from the base (n/N=8/4) and apex (n/N=6/4) of pre-pubertal female hearts and were superimposed for comparison. n = number of myocytes, N = number of hearts.

B: Summary of I_{NCX} densities at + 60 mV; I_{NCX} was higher at male base than apex myocytes and than base and apex I_{NCX} of female myocytes ($p < 0.05$). I_{NCX} was not statistically different at the base and apex of female myocytes (NS, $p = 0.58$).

Analysis of Inward and Outward I_{NCX} density from Pre-pubertal Myocytes

		N	Mean $\pm$ SD	Median (25–75% range)	P values
Male Base	Outward I _N CX	7	1.61 $\pm$ 0.9 [†]	1.54 (0.99 $\div$ 2.36) [†]	[†] 0.0005
	Inward I _N CX	7	-0.82 $\pm$ 1.0 [†]	-0.82 (-1.04 $\div$ -0.34)	[†] 0.014
Male Apex	Outward I _N CX	6	1.00 $\pm$ 0.5 [‡]	0.96 (0.67 $\div$ 1.40)	[‡] 0.001
	Inward I _N CX	6	-0.55 $\pm$ 0.6 [#]	-0.41 (-0.95 $\div$ -0.17)	[#] 0.58
Female Base	Outward I _N CX	8	0.40 $\pm$ 0.3 [†]	0.26 (0.21 $\div$ 0.58)	
	Inward I _N CX	8	-0.41 $\pm$ 0.6 [†]	-0.32 (-0.54 $\div$ 0.03)	
Female Apex	Outward I _N CX	6	0.31 $\pm$ 0.2 [‡]	0.35 (0.05 $\div$ 0.49)	
	Inward I _N CX	6	-0.50 $\pm$ 0.6 [#]	-0.40 (-0.72 $\div$ -0.10)	

N= number of hearts; for each heart and each voltage value, INCX density from different myocytes was averaged. The mean outward INCX was calculated from N x the averaged INCX values at +20 and 0 0 mV and the mean inward INCX was calculated from N x the averaged INCX at -80, -60, -40 and -20 mV. Unpaired Mann-Whitney U Test was applied to test for statistically significant differences of outward INCX at 0 and +20 mV. Female base vs. Male base: M=14 (7 male hearts X 2 voltage values), F=16 (8 female hearts X 2 voltage values), p=0.0005; Female apex vs. Male apex: M=12 (6 male hearts X 2 voltage values), F=12 (6 female hearts X 2 voltage values) p=0.001. Unpaired Mann-Whitney U Test was applied to inward INCX density measured at -80, -60, -40, and -20 mV; Female base vs. Male base: M=28 pairs (7 male hearts X 4 values), F=32 pairs (8 female hearts X 4 values), p=0.014; Female apex vs. Male apex: M=24 (6 male hearts X 4 values), F=24 (6 female hearts X 4 values), p=0.578