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Accentuated vagal antagonism paradoxically increases ryanodine receptor calcium leak in long-term exercised Calsequestrin2 knockout mice

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Abstract

BACKGROUND—Long-term aerobic exercise alters autonomic balance, which may not be favorable in heart rate (HR)–dependent arrhythmic diseases including catecholaminergic polymorphic ventricular tachycardia (CPVT) because of preexisting bradycardia and increased sensitivity to parasympathetic stimulation.

OBJECTIVE—The purpose of this study was to determine whether long-term exercise-induced autonomic adaptations modify CPVT susceptibility.

METHODS—We determined exercise-induced parasympathetic effects on HR, arrhythmia incidence, and intracellular sarcoplasmic reticulum (SR) Ca²⁺ leak in atrial (ACM) and ventricular (VCM) cardiomyocytes, in exercised (EX) calsequestrin knockout (CASQ2^{−/−}) mice, a model of CPVT.

RESULTS—Although 8-week treadmill running improved exercise capacity in EX CPVT mice, the incidence and duration of ventricular tachycardia also increased. HR variability analyses

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Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.hrthm.2017.10.008>.

revealed an increased high-frequency component of the power spectrum and root mean square of successive differences in R-R intervals indicating accentuated vagal antagonism during β -adrenergic stimulation resulting in negligible HR acceleration. In EX CASQ2^{-/-} VCM, peak amplitude of Ca²⁺ transient (CaT) increased, whereas SR Ca²⁺ content decreased. Aberrant Ca²⁺ sparks occurred at baseline, which was exacerbated with isoproterenol. Notably, although 10 μ M of the cholinergic agonist carbachol prevented isoproterenol-induced Ca²⁺ waves in ACM, CaT amplitude, SR Ca²⁺ load, and isoproterenol-induced Ca²⁺ waves paradoxically increased in VCM. In parallel, ventricular ryanodine receptor (RyR2) protein expression increased, whereas protein kinase A- and calmodulin-dependent protein kinase II-mediated phosphorylation of RyR2 was not significantly altered, which could imply an increased number of “leaky” channels.

CONCLUSION—Our novel results suggest that long-term exercise in CASQ2^{-/-} mice increases susceptibility to ventricular arrhythmias by accentuating vagal antagonism during β -adrenergic challenge, which prevents HR acceleration and exacerbates abnormal RyR2 Ca²⁺ leak in EX CASQ2^{-/-} VCM.

Keywords

Accentuated antagonism; Arrhythmia; Calcium; Catecholaminergic polymorphic ventricular tachycardia; Exercise; Vagal reflex

Introduction

Epidemiologic studies show that lifestyle improvements incorporating a healthy diet and regular physical activity can prevent cardiac disorders and lower mortality rates and complications from preexisting heart diseases.¹ However, long-term exercise alters autonomic balance extensively,² which may or may not be beneficial to all cardiac patients, especially to those susceptible to fatal arrhythmias, including catecholaminergic polymorphic ventricular tachycardia (CPVT). Hence, understanding how exercise modifies sympathovagal balance and how these autonomic adaptations affect intracellular sarcoplasmic reticulum (SR) calcium (Ca²⁺) leak is vital to determine whether exercise is a proactive, safe therapy for CPVT-prone hearts.

We previously demonstrated that Calsequestrin knockout (CASQ2^{-/-}) mice, a model of CPVT, are bradycardic and increasingly sensitive to parasympathetic stimulation.³ Faggioni et al⁴ demonstrated that low heart rate (HR) due to higher parasympathetic effects increases the incidence of CPVT in CASQ2^{-/-} mice. Although these findings identified an important role for parasympathetic stimulation in facilitating CPVT, the functional details are unknown.

The goal of this study was to determine the role of parasympathetic activity on HR during adrenergic stimulation in exercised (EX) CPVT mice and whether it affects intracellular SR Ca²⁺ leak. In addition to cardio-metabolic improvements, we studied (1) heart rate variability (HRV); (2) intrinsic HR; (3) vagal response during sympathetic activation in sedentary (SED) and EX wild-type (WT) and CASQ2^{-/-} mice; (4) intracellular Ca²⁺ cycling and ryanodine receptor (RyR2) Ca²⁺ leak in atrial (ACM) and ventricular (VCM) cardiomyocytes in the presence/absence of adrenergic and/or muscarinic stimulation; and (5)

protein expression profiles of SR Ca^{2+} handling proteins, and β -adrenergic receptor (β 1-AR) and muscarinic receptor type 2 (M_2R).

Methods

For an expanded Methods section, see the Supplemental Data.

Animals

All animal procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male WT and $\text{CASQ2}^{-/-}$ mice⁵ (age 2.5 months) of mixed background were randomly assigned to either the SED or EX group ($n = 30$).

Exercise training and testing

Aerobic interval training (AIT) protocol and exercise testing were designed based on previous studies with modifications.^{6,7}

Electrocardiographic Recordings

Surface electrocardiographic (ECG) traces, arrhythmia susceptibility, and vagal and sympathetic effects on HR were determined according to published protocols.^{5,8}

Confocal SR Ca^{2+} imaging

Confocal imaging of Ca^{2+} cycling in mouse ACM and VCM was performed according to our previous protocols.⁹ In some experiments, 100 nM isoproterenol (Iso) and/or 10/100 μM carbachol (CCh) in Tyrode solution were superfused.

Statistical analysis

Data are given as mean $\pm$ SEM and were analyzed with the Student *t* test/analysis of variance for multiple samples. $P < .05$ was considered significant for all results analyzed.

Results

AIT improves exercise capacity in EX $\text{CASQ2}^{-/-}$ mice

WT and $\text{CASQ2}^{-/-}$ mice successfully completed the 8-week AIT protocol ($n = 24$) (Figure 1A). While SED $\text{CASQ2}^{-/-}$ mice displayed lower exercise capacity compared to SED WT mice, after exercise training, EX WT and EX $\text{CASQ2}^{-/-}$ mice showed significant increases in maximum VO_2 levels (Figures 1B, and 1D–1F), maximum running speed, and time (Figure 1C) during the graded exercise test with comparable metabolic improvements (Supplemental Figures 1A and 1B).

Incidence and duration of ventricular tachycardia increase in EX $\text{CASQ2}^{-/-}$ mice

Analyses of baseline surface ECG recordings showed P-waves with significantly increased amplitude and decreased duration in SED and EX $\text{CASQ2}^{-/-}$ mice compared to respective WT groups (Figures 2A and 2B), whereas other ECG parameters were unaltered (see

Supplemental Table 1). Although atrial arrhythmias were not observed, catecholaminergic challenge induced a similar variety of abnormal ventricular rhythm patterns only in SED and EX CASQ2^{-/-} mice and not in SED and EX WT mice. Arrhythmias included premature ventricular complexes in trigeminy or bigeminy alternating between stretches of nonsustained, sustained, and bidirectional ventricular tachycardia (VT) (n = 6/6) (Figure 2C). Importantly, the total number of VT episodes and their maximum duration increased in EX CASQ2^{-/-} mice (Figures 2D and 2E).

HR acceleration is negligible in SED CASQ2^{-/-} and EX CASQ2^{-/-} mice during β -adrenergic stimulation

During β -adrenergic stimulation, HR acceleration was significantly higher in SED WT+Iso (+62 bpm) and EX WT+Iso (+103bpm) compared to baseline values, whereas HR in SED CASQ2^{-/-}+Iso (+31 bpm) and EX CASQ2^{-/-}+Iso (+27 bpm) mice were not different from their baseline values (Supplemental Table 2 and Figure 3A). However, similar to WT groups, ejection fraction significantly increased in SED CASQ2^{-/-}+Iso and EX CASQ2^{-/-}+Iso compared to baseline values (Figure 3B).

Heart weight-to-tibia length ratios increased significantly in both EX groups compared to their SED controls (Figure 3C). Atrial weight-to-tibia length ratios remained significantly higher in both SED CASQ2^{-/-} and EX CASQ2^{-/-} groups compared to SED and EX WT mice, respectively (Figure 3D), whereas ventricle weight-to-tibia length ratio significantly increased only in EX WT compared to SED WT mice (Figure 3E). Histologic examination of cardiac sections showed no signs of atrial or ventricular pathology and/or abnormal fibrotic remodeling in all 4 groups (data not shown).

AIT decreases sympathetic effects on HR in EX WT and EX CASQ2^{-/-} mice

Compared to their SED controls, average baseline HR in EX WT and EX CASQ2^{-/-} mice trended to decrease by 54 and 35 bpm, respectively, with no significant AIT-induced differences in intrinsic HR; however, intrinsic HR was significantly lower in SED and EX CASQ2^{-/-} mice compared to the WT groups (n = 10) (Figures 4A and 4B). Sympathetic effects on HR significantly decreased in the EX groups compared to their SED controls (Figure 4C), whereas vagal effects were significantly higher in EX CASQ2^{-/-} mice compared to EX WT mice (n = 8) (Figure 4D).

Vagal antagonism is robustly recruited during adrenergic stimulation in EX CASQ2^{-/-} mice resulting in negligible HR acceleration

Power values of the low-frequency component significantly increased during Iso challenge in EX WT+Iso, SED CASQ2^{-/-}+Iso, and EX CASQ2^{-/-}+Iso, indicating normal HR response to β -adrenergic stimulation (n = 9) (Figure 4E); however, the high frequency (HF) component, an indirect measure of parasympathetic responses, which trended to decrease during Iso treatment in other groups, paradoxically increased in EX CASQ2^{-/-} mice (Figure 4F). Average R-R intervals significantly decreased after Iso injection in SED WT, EXWT, and SED CASQ2^{-/-} mice, whereas there were no differences between baseline and Iso values in EX CASQ2^{-/-} mice, indicating negligible HR acceleration in response to Iso (Figure 4G). Root mean square of successive differences (RMSSD) of neighboring R-R

intervals were significantly lower in SED WT+Iso compared to SED WT and trended to decrease in EX WT+Iso and SED CASQ2^{-/-}+Iso, suggesting vagal withdrawal during Iso treatment (Figure 4H). However, average RMSSD significantly increased in EX CASQ2^{-/-}+Iso compared to SED CASQ2^{-/-}+Iso, indicating a predominance of vagal effects after AIT training. Thus, HRV analyses collectively indicate that β -adrenergic stimulation, which is known to trigger CPVT, also recruits higher vagal antagonism in EX CASQ2^{-/-} mice, which could contribute to negligible HR acceleration.

Enhanced SR Ca²⁺ release, decreased SR Ca²⁺ load, and increased Ca²⁺ sparks in EX CASQ2^{-/-} VCM

Confocal Ca²⁺ imaging revealed that the peak amplitude of cytosolic Ca²⁺ transient (CaT) significantly increased, while that of caffeine-induced CaT representing SR Ca²⁺ load, decreased both at baseline and with Iso in EX CASQ2^{-/-} VCM compared to SED CASQ2^{-/-} cells (Figures 5A–5C). We further examined whether AIT impacts Ca²⁺ spark mediated RyR2 Ca²⁺ leak in EX CASQ2^{-/-} VCM; Iso treatment increased Ca²⁺ spark frequency in both SED and EX CASQ2^{-/-} groups (Figure 5D). Of note, although EX CASQ2^{-/-} VCM showed significantly higher incidence of Ca²⁺ sparks even at baseline, arrhythmogenic waves during pacing intervals were observed only in SED CASQ2^{-/-}+Iso and EX CASQ2^{-/-}+Iso VCM (Figure 5E). After cessation of pacing, spontaneous Ca²⁺ waves developed significantly sooner at baseline in EX CASQ2^{-/-} VCM (Figure 5F). Moreover, reactive oxygen species levels, known to increase RyR2 Ca²⁺ leak, were not altered by exercise training in CASQ2^{-/-} VCM (Supplemental Figures 2A–2C).

Cholinergic stimulation paradoxically increases SR Ca²⁺ release and Ca²⁺ load in EX CASQ2^{-/-} VCM

Although activation of muscarinic receptor (MR) signaling with 10 μ M CCh decreased the peak amplitude of cytosolic CaT and SR Ca²⁺ load in EX WT VCM, unexpectedly, it increased both parameters significantly in EX CASQ2^{-/-} VCM (Figures 6B and 6C). Furthermore, contrary to SED CASQ2^{-/-}+Iso cells, 10 μ M CCh failed to decrease Iso-induced Ca²⁺ spark frequency and significantly increased diastolic Ca²⁺ waves (Figures 6D and 6E) with decreased time to spontaneous waves (Figure 6F) in EX CASQ2^{-/-}+Iso VCM. Moreover, whereas a higher dose of 100 μ M CCh was able to effectively prevent Iso-induced Ca²⁺ sparks in SED CASQ2^{-/-} cells, a significant number of aberrant Ca²⁺ sparks continued to occur in EX CASQ2^{-/-} VCM (Figure 6D). These novel data collectively show that, surprisingly, CCh-mediated MR activation enhances SR Ca²⁺ cycling at baseline and during adrenergic stimulation, does not decrease the number of Iso-induced Ca²⁺ sparks and further increases arrhythmogenic Ca²⁺ waves in EX CASQ2^{-/-} cells, indicating a paradoxical stimulatory role for muscarinic signaling in EX CASQ2^{-/-} ventricles. In parallel, Iso treatment significantly increased CaT amplitude in SED and EX WT and CASQ2^{-/-} ACM, whereas Ca²⁺ sparks and waves during pacing intervals increased only in EX CASQ2^{-/-} cells (Figures 7A–7D). Furthermore, in EX CASQ2^{-/-} ACM, 10 μ M CCh significantly decreased the peak amplitude of CaT and effectively prevented Iso-induced Ca²⁺ waves (Figures 7A, 7E, and 7G), while Ca²⁺ spark frequency also trended to decrease (Figures 7A and 7F).

Altered expression of SR proteins and autonomic receptors in EX hearts

Quantitative western blotting of atrial and ventricular homogenates showed that expression of SR proteins, including SERCA2a, CASQ2, Phospholamban, and DHPR2a was not significantly altered by AIT (Figures 8A and 8F). Importantly, M₂R expression was significantly increased only in EX CASQ2^{-/-} atrial samples (Figures 8A and 8C), whereas β 1-AR expression was similar in both tissues from SED and EX groups. Sodium-calcium exchanger (NCX) protein levels were significantly increased in EX WT atria and EX CASQ2^{-/-} ventricles (Figures 8A, 8B, 8F, and 8G). Total RyR2 expression was significantly increased in EX CASQ2^{-/-} ventricles (Figures 8F and 8H). Furthermore, phosphorylation at S-2808 (protein kinase A) was increased in EX CASQ2^{-/-} atria (Figures 8A and 8D) and EX WT ventricles (Figures 8F and 8I), whereas S-2814 (calmodulin-dependent protein kinase II) phosphorylation increased in both EX WT atria and ventricles relative to total RyR2 protein expression (Figures 8A, 8E, 8F, and 8J).

Discussion

Data from this study indicate that exercise-induced autonomic adaptations play a dual role in triggering CPVT in EX CASQ2^{-/-} mice by (1) decreasing HR and (2) causing a paradoxical increase in SR Ca²⁺ leak via the RyR2 in VCM. We found that despite catecholaminergic arrhythmias, CASQ2^{-/-} mice were able to exercise, resulting in significantly improved exercise capacity (Figure 1) and unaltered cardiac function (see Supplemental Table 2). Our findings are in keeping with previous reports, which also showed that CPVT mice and human patients are able to perform moderate-intensity exercise.^{6,10,11} In addition to absence of abnormal ventricular remodeling (Figure 3E), no difference in atrial weight, P-wave amplitude, and duration between SED and EX CASQ2^{-/-} mice indicates unaltered atrial structural and electrical remodeling after AIT (Figures 2A, 2B, and 3D, and Supplemental Table 1).³

The main goal of this study was to determine parasympathetic activity during β -adrenergic stimulation in EX CASQ2^{-/-} mice and whether it impacts CPVT. Baseline HR trended to decrease in EX mice after 8 weeks AIT (Figure 4A); sympathetic effects decreased in EX WT and EX CASQ2^{-/-} mice and vagal effects were significantly increased in EX CASQ2^{-/-} mice suggesting that both opposing branches could have contributed to decreasing baseline HR in EX CASQ2^{-/-} mice (Figures 4C and 4D). Moreover, in agreement with previous findings,¹² there were no significant differences in intrinsic HR in EX mice (Figure 4B), implicating autonomic modifications to be primarily responsible for the lower baseline HR in this study.

We found that sympathetic stimulation failed to accelerate HR in EX CASQ2^{-/-} mice, emphasizing critical differences in HR response to β -adrenergic stimulation between SED and EX CASQ2^{-/-} mice (Supplemental Table 2, and Figures 3A and 4G). Spectral analyses of HRV revealed that frequencies for power in the low-frequency range increased in EX CASQ2^{-/-}, similar to SED CASQ2^{-/-} after β -adrenergic stimulation (Figure 4E), suggesting that sympathetic response is present. However, contrary to SED CASQ2^{-/-} mice, β -adrenergic stimulation elicited an enhanced vagal response in EX CASQ2^{-/-} mice, which could have prevented HR acceleration (Figures 4F–4H). Based on previous findings that

implicate low HR in CASQ2^{-/-} mice in facilitating CPVT by widening the diastolic window during which intracellular SR Ca²⁺ leak can increase,⁴ our findings suggest that exercise-enhanced vagal responses might further increase CPVT susceptibility by debilitating robust HR acceleration. Importantly, our results indicate that β -adrenergic stimulation might simultaneously accentuate parasympathetic effects, particularly in EX CASQ2^{-/-} mice.

Such heightening of vagal antagonism during background sympathetic stimulation, also referred to as “accentuated antagonism,” has been previously described in both anesthetized and conscious animals and humans.^{13–15} Although the underlying mechanism in EX CASQ2^{-/-} mice is unknown, studies have shown that sympathetic stimulation can increase parasympathetic ganglia excitability¹⁶ or directly increase acetylcholine release at nerve terminals,^{17,18} thereby activating a reflex parasympathetic response. Furthermore, vagal activation has been strongly associated with exercise-induced arrhythmias, including atrial fibrillation. Interestingly, Schwartz et al^{19,20} have also demonstrated in patients with long QT syndrome type 1 that exercise-induced higher vagal reflexes can play a nongenetic modifier role in increasing risk for life-threatening arrhythmias. Our data indicate that chronotropic incompetence presenting as deficient HR acceleration during sympathetic activation becomes more apparent after exercise training in CPVT mice. This important finding highlights a preexisting sympathovagal imbalance, which could have been further altered after exercise training, resulting in disproportionately high vagal responses triggered by adrenergic challenges. Although it is unclear whether central or peripheral vagal activities caused the observed autonomic imbalance in EX CASQ2^{-/-} mice, since the dysfunctional CASQ2^{-/-} sinoatrial node (SAN) is increasingly sensitive to parasympathetic stimulation,³ we suggest that AIT-induced autonomic effects in CPVT could predominantly be SAN mediated. However, the unique interactions among autonomic signaling, ion currents, and the altered “Ca²⁺ clock” in EX CASQ2^{-/-} SAN myocytes in mediating accentuated antagonism have not yet been investigated.

Although the effects of vagal stimulation in opposing the β -adrenergic pathway are well known in atria and the SAN, parasympathetic effects in the ventricle are much more complex and unclear.²¹ In keeping with our *in vivo* data, which showed that sustained VT episodes increase during β -adrenergic stimulation in EX CASQ2^{-/-} mice (Figures 2C–2E), we also found that SR Ca²⁺ cycling is significantly modified in EX CASQ2^{-/-} VCM characterized by higher SR Ca²⁺ release, decreased SR Ca²⁺ load, and increased aberrant Ca²⁺ sparks even at baseline compared to SED CASQ2^{-/-} controls (Figures 5A–5D). Notably, abnormal Ca²⁺ sparks and waves increased during β -adrenergic stimulation, which might also account for the decrease in SR Ca²⁺ load in these cells, whereas spontaneous waves appeared sooner in EX CASQ2^{-/-} VCM (Figures 5D–5F). Importantly, we found for the first time that, contrary to the known inhibitory effects of cholinergic stimulation, 10 μ M CCh paradoxically increased SR Ca²⁺ release and load only in EX CASQ2^{-/-} VCM, whereas both parameters decreased in EX WT cells as expected (Figures 6B and 6C). Furthermore, CCh failed to decrease Iso-induced Ca²⁺ sparks and increased the incidence of spontaneous waves in EX CASQ2^{-/-} VCM compared to SED CASQ2^{-/-} cells, suggesting that MR signaling might have enabled proarrhythmic Ca²⁺ wave formation during β -adrenergic stimulation in these cells (Figures 6D and 6E). However, a higher dose of 100 μ M CCh effectively prevented abnormal Ca²⁺ waves but not sparks in EX CASQ2^{-/-} VCM,

indicating a dose-dependent effect of MR stimulation on RyR2 activity (Figures 6D and 6E). In contrast, although Ca^{2+} sparks and waves were higher during Iso treatment in EX CASQ2^{-/-} ACM (Figures 7C and 7D), muscarinic activation with 10 μM CCh completely prevented aberrant Ca^{2+} waves (Figure 7G), potentially due to higher levels of M₂R in EX CASQ2^{-/-} atria (Figures 8A and 8C). Taken together, these data suggest that MR activation could mediate different effects on Iso-induced RyR2 activity in EX CASQ2^{-/-} atria vs ventricle and facilitate CPVT by increasing abnormal Ca^{2+} wave formation only in EX CASQ2^{-/-} VCM.

Although intriguing, the mechanism causing the stimulatory effect of MR activation on SR Ca^{2+} cycling in EX CASQ2^{-/-} VCM is unknown. In normal hearts, cholinergic stimulation has been shown to directly or indirectly affect ion channel activity mainly by modulating cAMP levels, thereby potentially altering phosphorylation of multiple targets.²² However, its effects on SR Ca^{2+} cycling in the context of a “leaky” RyR2 have never been studied. Complementary coactivation of both autonomic limbs are known to modulate HR, but whether such interactions affect SR Ca^{2+} cycling in the VCM is unclear.²³ The stark difference in the actions of CCh on RyR2 activity between SED and EX CASQ2^{-/-} VCM indicates distinct exercise-induced modifications of the cholinergic signaling pathway and/or how it interacts with the β -adrenergic pathway to modulate SR Ca^{2+} leak. Similar to previous findings, AIT did not alter M₂R and β 1-AR protein expressions in both EX WT and EX CASQ2^{-/-} ventricles,²⁴ whereas NCX expression was increased only in EX CASQ2^{-/-} ventricles (Figures 8F and 8G). Interestingly, a previous study found that the reverse mode of NCX can also mediate CCh-enhanced Ca^{2+} cycling and associated positive inotropism.²⁵ Although we did not examine NCX activity in this study, increased NCX expression in EX CASQ2^{-/-} ventricles could increase VT as well as enable an alternate mechanism potentiating the stimulatory effect of CCh. Further studies are warranted to determine the mechanistic details of how cholinergic stimulation increases RyR2 activity in EX CASQ2^{-/-} VCM and especially, how it contributes to CPVT.

Phosphorylation at multiple sites on the RyR2 is known to play major roles in regulating SR Ca^{2+} release and promoting abnormal Ca^{2+} leak during adrenergic stimulation.²⁶ The observed increase in Ca^{2+} release could not be attributed to alterations in RyR2 phosphorylation at S-2808 and S-2814 sites in EX CASQ2^{-/-} ventricles (Figures 8F, 8I, and 8J); however, EX WT ventricles showed increased levels of both protein kinase A- and calmodulin-dependent protein kinase II-dependent phosphorylation, underscoring a critical difference in exercise-induced RyR2 phosphorylation between EX WT and EX CASQ2^{-/-} ventricles. Alternately, total RyR2 protein expression increased in EX CASQ2^{-/-} ventricles (Figures 8F and 8H), which could imply a higher number of “leaky” RyR2s and account for the increased Ca^{2+} sparks and decrease in time to wave formation. In contrast, RyR2 phosphorylation at S-2808 was increased in EX CASQ2^{-/-} atria with unaltered total RyR2 expression (Figures 8A and 8D), indicating that different mechanisms could promote RyR2 Ca^{2+} leak in EX CASQ2^{-/-} atria vs ventricles.

Study limitations

Differences in SR Ca^{2+} cycling between rodents vs humans may limit extrapolation of our findings to human CPVT patients. We and others have previously demonstrated adrenergic arrhythmias in several CPVT mouse models under isoflurane anesthesia confirming RyR2-mediated Ca^{2+} leak and functional, albeit mildly decreased, autonomic activity despite anesthesia.^{3,13,20,27} Furthermore, previous studies have shown that although isoflurane can dose dependently decrease both sympathetic and parasympathetic effects, their mutual cross-talk facilitating accentuated antagonism between the 2 branches is intact even in anesthetized animals and humans.¹³ Because ECGs were recorded under the lowest dose of inhaled isoflurane anesthesia (1.25–1.5% v/v) recommended for minimal cardiac function depression in mice,²⁸ and study parameters were statistically tested between and within control and experimental groups, we are confident that despite the undisputable effects of low-dose isoflurane anesthesia, our findings are rigorous and reveal sympathovagal imbalance inherent only to CPVT mice.

Conclusion

During β -adrenergic challenge, parasympathetic antagonism is significantly heightened in EX CASQ2^{-/-} mice, which impacts arrhythmogenesis by 2 mechanisms: (1) prevents HR acceleration; and (2) exacerbates intracellular SR Ca^{2+} cycling abnormalities in VCM. Although findings from mouse models may not be directly applicable to human CPVT patients, our data indicate that mild, recreational physical activity could be harmless and even beneficial, but also emphasize caution and regular HRV profile monitoring for exercising CPVT patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Exercise capacity in EX WT and CASQ2^{-/-} mice. **A:** Aerobic exercise training protocol. Summary graphs of maximum VO₂ (**B**) and corresponding maximum speed at the end of graded exercise test (**C**) (numbers inside the columns indicate maximum running times during the test), VO₂ (**D**), VCO₂ (**E**), and respiratory exchange ratio (RER) (**F**) (RER = VCO₂/VO₂). n = 9–12. **P* < .05. EX = exercised; SED = sedentary; WT = wild-type; CASQ2^{-/-} = Calsequestrin knockout.

Figure 2.

P-wave morphology and arrhythmia incidence in SED and EX CASQ2^{-/-} mice. Summary graphs showing P-wave amplitude (**A**) and P-wave duration (**B**), representative ECG traces (**C**), VT episodes (**D**), and average duration of VT episodes (**E**). $n = 8$. * $P < .05$). caff = caffeine; epi = epinephrine; PVC = premature ventricular complex; VT = ventricular tachycardia; other abbreviations as in Figure 1.

Figure 3.

Heart rate responses and morphometrics in SED and EX WT and CASQ2^{-/-} mice. Summary data showing average heart rate changes in response to Iso (**A**) and ejection fraction (**B**). n = 6. **P* < .05. **C**: Heart weight to tibia length ratio. **D**: Atria weight to tibia length ratio. **E**: Ventricle weight to tibia length ratio. n = 8. **P* < .05. Iso = isoproterenol; other abbreviations as in Figure 1.

Figure 4.

Autonomic effects on HR and HR variability analyses in SED and EX WT and CASQ2^{-/-} mice. Summary data of baseline HR (A), intrinsic HR after complete autonomic blockade (B), sympathetic effects on HR (change in HR between atropine followed by propranolol injections) (C), and vagal effects (change in HR between propranolol followed by atropine injections) on HR (D). Power spectral analyses of sinus beats from ECG recordings at baseline and +Isoproterenol. Summary data of frequencies for power in the low-frequency (LF) range (E), high-frequency (HF) range (F), average R-R intervals (G), and root mean

square of the successive differences (RMSSD) of neighboring R-R intervals (**H**). $n = 9$. $*P < .05$. HR = heart rate; other abbreviations as in Figure 1.

Figure 5.

Sarcoplasmic reticulum Ca²⁺ cycling parameters and RyR2 activity in SED and EX WT and CASQ2^{-/-} ventricular myocytes. **A:** Representative line-scan images $\pm$ ISO. Summary data showing peak amplitude of CaT (**B**), caffeine-induced CaT (representing SR Ca²⁺ load) (**C**), Ca²⁺ spark frequency (**D**), diastolic Ca²⁺ waves per cell (**E**), and time to spontaneous Ca²⁺ wave after pacing was stopped (**F**). $n = 3$ mice, 50–69 cells/group. * $P < .05$). CaT = calcium transient; ISO = isoproterenol; other abbreviations as in Figure 1.

Figure 6.

Effect of muscarinic receptor stimulation on sarcoplasmic reticulum Ca^{2+} cycling parameters and RyR2 Ca^{2+} leak in SED and EX WT and $\text{CASQ2}^{-/-}$ ventricular myocytes.

A: Representative line-scan images +ISO, 10 μM CCh, or 100 μM CCh. Summary data showing peak amplitude of calcium transient (CaT) (B), caffeine-induced CaT (C), Ca^{2+} spark frequency (D), full Ca^{2+} waves per cell (E), and time to spontaneous Ca^{2+} wave after stimulation was stopped (F). $n = 3$ mice, 6–12 cells per treatment/heart. $*P < .05$. CCh = carbachol; other abbreviations as in Figures 1 and 5.

Figure 7.

Sarcoplasmic reticulum Ca²⁺ cycling parameters and effect of muscarinic receptor stimulation in SED and EX WT and CASQ2^{-/-} atrial myocytes. **A:** Representative line-scan images at baseline, +ISO, and 10 μM CCh. Summary data of peak amplitude of CaT (**B**), Ca²⁺ spark frequency (**C**), Ca²⁺ waves per cell (**D**), and after treatment with 10 μM CCh (**E**–**G**). n = 3 mice, 8–19 cells per treatment/heart. **P* < .05. CCh = carbachol; other abbreviations as in Figures 1 and 5.

Figure 8.

Expression of SR Ca²⁺ handling proteins and autonomic receptors in atria and ventricles of SED and EX WT and CASQ2^{-/-} mice. Representative immunoblots of SR Ca²⁺ handling protein expression and autonomic receptor expression normalized to α-sarcomeric actin levels in atria (A) and ventricles (F) from the same hearts. Summary data of protein expression: in atria (B–E) and ventricles (G–J). EX values are expressed as percentage of their SED control values. n = 5 per group. *P < .05. NCX = sodium-calcium exchanger; SR = sarcoplasmic reticulum; other abbreviations as in Figure 1.