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Alpha blockade potentiates CPVT therapy in calsequestrin-mutant mice

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Abstract

Background—Spontaneous calcium release evoking delayed after-depolarization is believed to cause CPVT, a lethal human arrhythmia provoked by exercise or emotional stress. Beta-adrenergic blockers are the drug of choice, but fail to achieve complete arrhythmia control in some patients. These individuals often require flecainide, device implantation and/or sympathetic denervation.

Objective—To optimize the arrhythmia therapy by pharmacological inhibition of the sympathetic nervous system in the CASQ2$^{\Delta/\Delta}$ mouse model of CPVT2.

Methods—A heart telemetry device was implanted for continuous ECG recording at rest and during provocation testing. Calcium transients and abnormal calcium release were studied in cardiomyocytes isolated from adult mice. Adrenergic receptor expression was determined by western blotting and confocal microscopy.

Results—Adult CASQ2$^{\Delta/\Delta}$ mice suffer from complex ventricular arrhythmia at rest and ventricular tachycardia during treadmill exercise and after epinephrine injection. Beta adrenergic blockers, propranolol and metoprolol attenuated arrhythmia at rest but not after stress. Reserpine

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had no efficacy in controlling arrhythmia. Agents with alpha blocking activity, phentolamine or labetalol, abolished both exercise and epinephrine-induced arrhythmia. To the contrary, injection of alpha adrenergic agonist phenylephrine reproducibly provoked VT. Isolated cardiomyocytes from CASQ2$^{\Delta/\Delta}$ mice had delayed calcium release waves upon exposure to sympathetic agonists which was abolished by phentolamine. Hearts of calsequestrin mutant mice expressed more alpha adreno-receptor1 compared to controls ($p<0.05$).

Conclusions—We identified a contribution of alpha adrenergic pathway to pathogenesis of catecholamine-induced arrhythmia. Alpha blockade emerges an effective therapy in the murine model of CPVT2 and should be tried in humans resistant to beta blockers.

Keywords

CPVT; calcium; calsequestrin; mouse model; arrhythmia; alpha adrenergic receptor; sympathetic blocker

Introduction

Catecholaminergic polymorphic ventricular tachycardia(CPVT) is a lethal human arrhythmia provoked by exercise or emotional stress[1-3]. CPVT is caused by mutations in genes that regulate intracellular Ca^{+2} homeostasis. Patients suffering from CPVT are at risk of ventricular fibrillation, seizures and sudden death triggered by exercise or emotional stress[2,4,5].Two principal types of gene mutations cause the disease: heterozygous defects in the cardiac ryanodine receptor gene (*RYR2*) cause the autosomal dominant CPVT1, while homozygous mutations in the cardiac calsequestrin (*CASQ2*) cause the autosomal recessive CPVT2[3, 6]. Calsequestrin is a sarcoplasmic reticulum (SR) protein which together with triadin and junction is essential for regulation of Ca^{+2} induced Ca^{+2} release through the ryanodine channel[7]. CASQ2 defects causing CPVT2 may be missense such as D307H[6], which are assumed to impair the conformational changes occurring during Ca^{+2} binding and the ability to regulate Ca^{+2} transfer to the cytosol through the ryanodine channel or null-allele mutations[8]. We have previously described mouse models recapitulating the human phenotype for CPVT2. These gene-targeted mice are homozygous for either the D307H mutation (CASQ2$^{D307H/D307H}$) or represent a CASQ2 knock-out (CASQ2$^{\Delta/\Delta}$). Polymorphic and bidirectional VT may be provoked by physical or pharmacological stress[9-11]. The phenotype of CASQ2$^{D307H/D307H}$mice was attributed mainly to the degradation of the defective protein leading to a nearly complete protein deficiency, despite preserved mRNA levels[10]. We also observed some difference in arrhythmia severity between adult CASQ2$^{D307H/D307H}$ and CASQ2$^{\Delta/\Delta}$ mice and a decreased responsiveness to drug therapy in the latter[9].

Beta-adrenergic blockers are the therapy of choice for human CPVT but they fail to achieve complete arrhythmia control in some patients[9, 11].Such individuals often require additional drugs, device implantation and/or sympathetic denervation.

The sympathetic nervous system plays a crucial role in the regulation of cardiac function. Norepinephrine (NE) released from sympathetic neurons innervating the heart affects the heart rate, cardiac contractility, blood flow, substrate utilization and mediates

cardiomyocytes hypertrophy. The effects of NE are mediated by nine different receptors (α1A-, α1B-, α1D-, α2A-, α2B-, α2C, β1-, β2- and β3). These receptors are part of a larger superfamily of G-protein coupled receptors that mediate the effects of hormones and neurotransmitters[12, 13].

In this study we attempted to optimize CPVT therapy by targeting several components of the autonomic nervous system. We hereby report that alpha adrenergic receptor blockers protect against arrhythmia and abolish abnormal calcium release in cardiomyocytes fromCASQ2 knockout mice.

Methods

Animals

The study was approved by the Institutional Committee for Animal Care and Use 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). Experiments were carried out on gene targeted C57BL/6 mice modeling CPVT2[10], homozygous for off-frame exon 9 deletion ($CASQ2^{\Delta/\Delta}$) or D307H mutation ($CASQ2^{D307H/D307H}$) in comparison to age-matched wild type (WT) mice. Mice were maintained and bred in a pathogen-free facility on regular rodent chow with free access to water and 12-hour light and dark cycles.

Unless otherwise stated, experiments were performed in adult (6-8 month old) male mice.

In vivo Pharmacological Testing

$CASQ2^{\Delta/\Delta}$ mice were implanted with a telemetry device (DSI St. Paul MM, device weight 3.8g) as previously described[14]. Provocation testing was performed at baseline and 15 min after IP drug injection of each drug (Table 1). A stock solution of Reserpine (MP Biomedicals) was prepared by diluting 4mg/ml in DMSO(Sigma). Mice were injected 0.5 μg/g of the primary stock. Provocation testing was repeated 24 hours after reserpine looking for the delayed effect of this drug. Phentolamine (Novartis) and labetalol (Trandate, Perrigo Israel Pharmaceuticals Ltd.) were used in doses previously tested in mice[15].

Arrhythmia was studied with heart rhythm telemetry at rest, during treadmill exercise (Exer-6M, Columbus Instruments, OH, USA) and after IP injection of epinephrine 0.1mg/kg (Teva, Israel). In brief, mice were forced to exercise on a rodent treadmill, gradually increasing the speed up to a maximum of 15 m/min. Then, after 3 minutes of rest, mice were injected with epinephrine, followed by additional 5 minutes of telemetric recording. Ventricular tachycardia (VT) was defined as four or more consecutive ventricular beats. All other ventricular arrhythmias, i.e. premature beats, ventricular bigeminy, couplets and triplets were all defined as premature ventricular contractions (PVCs). Animal expressing CPVT was defined by developing VT (either sustained or non-sustained) at rest, during exercise or pharmacological stress [9]

Experiments with phenylephrine

Phenylephrine 1mg/g(West-Ward Pharmaceutical, Eatontown, NJ, USA) or saline was injected IP to 3-month old mice following 10 minutes of recording at rest. These young

animals do not have arrhythmia under resting conditions. An ECG recording was initiated 5 min after the injection and continued for another 10 minutes. On the next day mice were pretreated by IP verapamil (2.5 mg/g, Abbott, IL, USA) prior to phenylephrine.

Cardiomyocyte isolation from adult mice and intracellular Ca^{+2} measurements

Adult ventricular cardiomyocytes were isolated from $CASQ2^{\Delta/\Delta}$ mice using a Langendorff isolated heart perfusion and enzymatic digestion as previously described[16]. Cells were incubated at 37°C in 2% CO_2 until used. $[Ca^{2+}]_i$ from individual adult cardiomyocytes was measured as above. Measurements were performed in electrically paced cells at 1 Hz via platinum electrodes, under baseline conditions, with pharmaceutical agents isoproterenol 10 μM (Monico Spa, Venice), norepinephrine 10μM (Hospira, Lake Forest, IL) and phentolamine 10μM, or their combination.

Intracellular free calcium $[Ca^{+2}]_i$ from individual adult cardiomyocytes was measured using the indicator indo-1-AM under a Zeiss epi-fluorescent inverted microscope (Zeiss, Germany). All the measurements were made on the day of heart isolation. Adult cardiomyocytes were incubated with 5μM indo-1-AM and 3μM pluronic acid for 30 min in glucose-enriched Tyrode's solution at 25°C. After incubation, the cells were rinsed with glucose-enriched Tyrode's and transferred to a chamber (RC-47FSLP Warner Instruments) on the microscope. Calcium measurements were made in cells in suspension. Indo-1 loaded cells were exited at 340 nm and the emitted light then split by a dichroic mirror into two photomultipliers (Hamamatsu, Japan), with input filters at 410 and 490 nm for indo-1. The fluorescence ratio (R) of 410/490 nm, which is proportional to $[Ca^{+2}]$i, was input to the SAMPLE program written by Dr. D. Kaplan, the Biological Institute, Ness-Ziona, Israel. Analysis was performed at 25°C.

Protein extraction and Western blot analysis

Proteins were extracted from frozen hearts and separated as previously described[14]. Electro-blotting to Hybond-C Extra membranes (Habersham, USA) was followed by incubation with rabbit anti-alpha 1 adrenergic receptor antibodies (1:400), (Acris Antibodies, Germany). For secondary detection, we used DyLight800 goat anti-rabbit (Pierce Biotechnology, Thermo Fisher Scientific, town, USA) (1:10000). Reactive bands were detected and quantified by the Odyssey® Infrared Imaging System (Li-Cor Biotechnology, town, USA).

Immunostaining of isolated cardiomyocytes

Fresh isolated cardiomyocytes were added to 10μl laminin (Sigma, town, Germany) on a cover glass and were fixed with 100% methanol (Bio-Labs, town Israel) incubation for 10 min. Cells were washed with PBS (Sigma, Germany) and the membrane was perforated with PBS containing triton X-100 (Sigma, Germany).Blocking was performed with PBS containing 5% BSA (Sigma, Germany) for 1 hour and samples were incubated overnight with primary antibodies, either beta 1 adreno-receptor (rabbit polyclonal antibody 1:100, SC-568, CA USA) or alpha 1 adrenergic receptor (rabbit polyclonal 1:1000, Acris Town, Germany). Afterwards cells were incubated for 2 hours at room temperaturewith1:1500 secondary antibody, goat anti-rabbit polyclonal IgG- H&L (FITC,ABCAM, Cambridge,

UK) and with Hoechst (Invitrogen, Paisley, UK) for 10 min. The cells were visualized and photographed using a Leica TCS SP5 Confocal Imaging System (Leica Microsystems, Wetzlar, Germany) and x63 oil objectives.

Statistical analyses were performed when appropriate using Student's t or Fisher's exact test. Data are presented as mean± SD and statistical significance was accepted at $p<0.05$.

Results

In vivo studies

Adult CASQ2$^{\Delta/\Delta}$ mice, implanted with the telemetry device, underwent provocation testing at baseline and after drug injection to evoke CPVT (Table 1).

All mutant mice studied in this experiment suffered from severe arrhythmia including VT at baseline. Beta adrenergic blockers were insufficiently effective against stress-induced VT irrespective of 1 selectivity. They prevented sustained ventricular tachycardia but not VPCs or non sustained VT.

Since patients that do not respond to β-blockers are treated by sympathetic denervation[17], we tried to induce sympathectomy by targeting sympathetic nerve endings with reserpine[17]. Unfortunately, CASQ2$^{\Delta/\Delta}$ mice did not respond to this treatment and all had VT during the provocation testing immediately or 24 hours after drug administration.

The non-selective α adrenergic blocker phentolamine had a pronounced antiarrhythmic efficacy. Because any pharmacological therapy for human CPVT should include beta blockade, we proceeded to experiment with the combined +1blocker, labetalol (having a 'β to α1' antagonism ratio of 3:1). This drug prevented VT in a dose-dependent fashion (Table 1, Fig 1).

To test if isolated α adrenergic stimulation would induce arrhythmia in CASQ2$^{\Delta/\Delta}$ mice we injected young animals (which do not have arrhythmia at rest) with a α1 agonist, phenylephrine. Mice (n=3) developed VT after being treated with phenylephrine but not with saline vehicle (Fig 2). This arrhythmia could be abrogated by pretreatment with verapamil which was previously shown by us to prevent CPVT in CASQ2 mutant mice[9].

Studies in isolated cardiomyocytes

Calcium transients were compared between WT and CASQ2$^{\Delta/\Delta}$ cardiomyocytes on baseline, after exposure to norepinephrine and to phentolamine (Table 2). WT transients had a higher amplitude and ascent rate at baseline. The descent rate followed a similar trend but did not reach statistical significance. An adrenergic agonist noradrenalin significantly augmented the transient in the WT. The area under curve representing the total systolic calcium release was significantly higher in WT cells compared to CASQ2$^{\Delta/\Delta}$ at baseline (24 23 vs. 16 ±10 unit, p=0.0012) and after noradrenaline (40 ±39 vs. 15 ±8, p=0.014). In CASQ2$^{\Delta/\Delta}$cells noradrenaline did not affect the parameters of initial calcium release but often triggered a second wave of delayed calcium release which was never seen in the WT (Figure 3). The blocker phentolamine, on its own, had no significant effect on transient parameters.

However it did attenuate the physiological and the pathological effects of norepinephrine on WT and CASQ2$^{\Delta/\Delta}$cells, respectively.

After documenting the protection of blockade against abnormal calcium release on combined (+) adrenergic stimulation with norepinephrine we examined if blockade could protect from calcium irregularities induced by a selective adrenergic agonist. As illustrated in Figure 4, Isoproterenol induced repetitive delayed calcium release, severely prolonging the transient in CASQ2$^{\Delta/\Delta}$cardiomyocytes. Phentolamine reproducibly abolished isoproterenol-induced abnormal calcium release in all cells tested.

Adrenergic alpha 1 (ADRA1) receptor expression in the heart of CASQ2 mutant mice

ADRA1proteinlevels were assessed in hearts of 14-16-week-old WT or either CASQ2$^{\Delta/\Delta}$ or CASQ2$^{D307H/D307H}$ mutant mice. The postsynaptic 1adreno-receptor was significantly elevated in total heart homogenates from either CASQ2 mutant mouse compared to the WT (Fig 5).

To further study cardiac alpha receptor expression, we also examined isolated adult CASQ2$^{\Delta/\Delta}$ cardiomyocytes by immunofluorescence. Confocal microscopy imaging confirmed ADRA1 immunostaining of isolated cardiomyocytes (Fig 6). As could be expected, receptor expression appeared to be lower compared to the expression of beta adrenergic receptors.

Discussion

We used mice with CASQ2 knock-out as a model for the optimization of CPVT therapy. The recessively inherited CPVT2 is associated with early disease onset, severe arrhythmia, and adverse prognosis[1, 8].The principal therapies for CPVT are phenotype driven and include avoiding exercise and, beta adrenergic blockade. The response to beta-blockers is incomplete and often declines with time because of an escape phenomenon[3,18]. The options in unresponsive patients include additional drugs - primarily flecainide, implanting a defibrillator (ICD), and sympathetic nervous denervation[17]. Although very effective in mice, calcium channel blockers have a limited benefit in humans, even when combined with beta adrenergic blockers. CaMKII inhibition and attenuating the degradation of mutant protein have shown promising results *in vitro* but may not yet be applied in clinical practice. Given the success of sympathetic denervation we examined whether drugs affecting the adrenergic pathway have a protective effect against CPVT.

Pharmacological testing conducted in mice and isolated cardiomyocytes identified a novel approach to treat CPVT. Alpha sympathetic blockade alone or alpha blocker combined with a beta blocker effectively eliminate arrhythmia in CASQ2 knockout mice (Table 1, Fig 1) and prevent abnormal calcium release in isolated cardiomyocytes from the affected animals (Table 2, Fig 3).

Administration of the alpha adrenergic agonist phenylephrine induced typical arrhythmia in CASQ2$^{\Delta/\Delta}$ mice, confirming the cause-and-effect relationship between alpha receptor activation and CPVT (Fig. 2).Importantly, arrhythmia could be abolished by verapamil and

was not present in CASQ2$^{\Delta/\Delta}$ mice injected with saline. Reproducing the protective effect of alpha blockade in isolated cardiomyocytes indicates a postsynaptic mechanism of action. Confocal imaging of isolated murine cardiomyocytes (Fig. 6) confirmed a low-level expression of the alpha adrenergic receptor on the sarcolemma. Although alpha receptors are less abundant compared to beta receptors, their activation has a major role in causing CPVT in mice. Interestingly, there was an increase in adreno-receptor expression in CASQ2 knockout or missense mutant mice (Fig. 5), suggesting that increased receptor responsiveness to sympathetic stimulation could contribute to arrhythmia susceptibility. Table 2 provides some insights as to the role played by alpha blockade in calcium transients. Noradrenaline increased the amplitude, the rise and decay rates of calcium transients in the wild type but not in the CASQ2$^{\Delta/\Delta}$ cardiomyocytes. While the initial phase of CASQ2$^{\Delta/\Delta}$ transients was not affected, some of them were followed by abnormal calcium release waves which never occurred in the wild type (Fig. 3). Phentolamine had no effect on calcium transient parameters under resting conditions. It attenuated the physiological changes induced by noradrenaline in wild-type cardiomyocytes and the pathological waves in cells from CPVT mice. Ability of phentolamine to prevent abnormal calcium release caused by pure beta adrenergic stimulation (Fig. 4), suggesting that these 2 pathways interfere at the post-receptor level.

Alpha adrenergic signaling affects vascular tone, cardiac function and metabolism. Chronic activation of myocardial alpha receptors leads to cardiac hypertrophy, increased energy expenditure followed by apoptosis and worsening heart failure ,produces detrimental effects like cardiac hypertrophy[13, 19]. On the other than, short-term activation may be cardio protective and even mediate preconditioning[20, 21].The role of alpha adrenergic signaling in CPVT and its relative importance in humans as compared to mice is not yet clear. The clinically documented antiarrhythmic effect of sympathetic denervation (on top of beta blockade) suggests that alpha adrenergic innervation does play a pathogenic role. In our study reserpine was ineffective, but this drug, despite causing “chemical sympathectomy” by depleting a neurotransmitter from nerve endings cannot neutralize circulating catecholamines.

Because alpha stimulation had no apparent interaction with beta receptor, PKA activation, L-type or ryanodine channel, we hypothesized that its activity may be mediated through phospholipase C and stimulation of the IP3 (inositol triphosphate) receptor in the perinuclear sarcoplasmic reticulum[4]. Intracellular Ca^{2+} stores include SR and the cisternae surrounding the nuclear envelope. The junctional SR is located in the proximity of t-tubules, L type calcium channels and contains the ryanodine receptors and the SR Ca^{2+} pump (SERCA). The free [Ca^{2+}] inside the perinuclear stores can be ~ 1mM but is not directly involved in excitation-contraction coupling. IP3 is the primary Ca^{2+} release channel in the smooth muscle, but even cardiac myocytes use local Ca^{2+} release from the perinuclear stores via IP3 receptors/channels in response to neurohumoral stimuli. The channel agonist IP3 is generated by phospholipase C cleavage of membrane phosphatidyl inositol bisphosphate (PIP_2) into IP3 and 1,2- diacylglycerol. IP3-induced Ca^{2+}sparks have lower amplitude and longer rise and descent times. In the ventricular myocyte there are about 50× less IP_3 receptors than RyR2 receptors and their location is mainly in the perinuclear envelope and not in the subsarcolemmal SR[22]. Yet, junctional SR appears to be contiguous with the

nuclear envelope in cardiomyocytes. While not directly participating in calcium induced calcium release, opening of IP_3channels may increase the basal Ca^{2+} level in the sarcoplasm thus affecting the susceptibility to arrhythmia in CPVT mice[23].

IP3 inhibitor 2APB was shown to prevent abnormal Ca^{2+} release in cardiomyocytes from the diabetic heart[24]. We have tried, so far without success, to attenuate stress induced arrhythmia in $CASQ2^{\Delta/\Delta}$ mice using parenteral 2APB (n=5, data not shown).

Alpha adreno-receptor blockers do not have an established role in treatment of human arrhythmia. Experimental studies suggest that alpha receptors modulate electrophysiological properties of the ventricular myocardium[25] and are involved in ventricular arrhythmia of ischemia reperfusion, catecholamine stimulation and heart failure[26, 27], as well as neutrally mediated atrial arrhythmia[28]. Our study demonstrates the presence of alpha adrenergic activity in the cardiomyocytes and favors the potential use of alpha blocking agents in catecholamine- induced arrhythmia.

Nonselective -blockers, propranolol and the peripherally acting nadolol[18,29] are the most commonly used agents in CPVT. While very high concentrations of propranolol might produce some alpha-receptor blockage, nadolol is devoid of this activity [30]. Reserpine, depleting the sympathetic nerve endings was ineffective in this study but might be tried in combination with beta blockade to antagonize circulating catecholamines. Labetalol comprises 1 with 2 and 1 blocking activity, thus closely mimicking sympathetic denervation by pharmacological means. That makes labetalol an excellent candidate to treat resistant CPVT cases and to avoid invasive procedures such as left cardiac sympathetic denervation and possibly ICD implantation.

Conclusions

The pathway by which alpha adreno-receptor stimulation participates in CPVT pathogenesis warrants further investigation. Another question is whether this result is applicable to the more common form of the disease, CPVT1. While the exact mechanism of protection by alpha blockers remains unclear, their effect against CPVT2 in a genetic murine model, which recapitulates the human disease, is rather remarkable. If reproduced in human studies, it may become an important non-invasive therapeutic option for CPVT patients.

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Abbreviations

ADRA	alpha adrenergic receptor
$CASQ2^{\Delta/\Delta}$	homozygous calsequestrin knock-out mice
$CASQ2^{D307H}$	mice homozygous for D307H calsequestrin mutation
CPVT	Catecholaminergic polymorphic ventricular tachycardia

IP	intraperitoneal
PVCs	premature ventricular contractions
SR	sarcoplasmic reticulum
VT	ventricular tachycardia
WT	wild type

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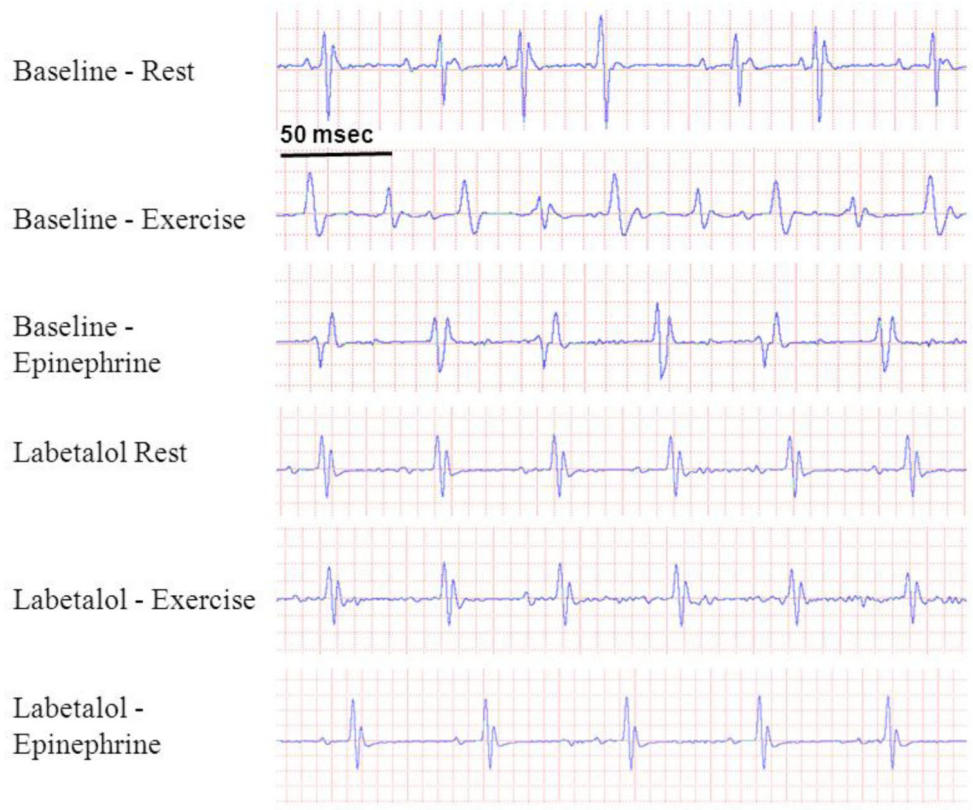

Figure 1.

Representative ECG traces from an 8 month old CASQ2$^{\Delta/\Delta}$ mouse undergoing provocation testing at baseline and after injection of labetalol. Sinus rhythm with multiple ventricular premature beats was recorded at rest, bidirectional VT after exercise and polymorphic VT after adrenaline. Labetalol evoked sinus bradycardia and eliminated the arrhythmia.

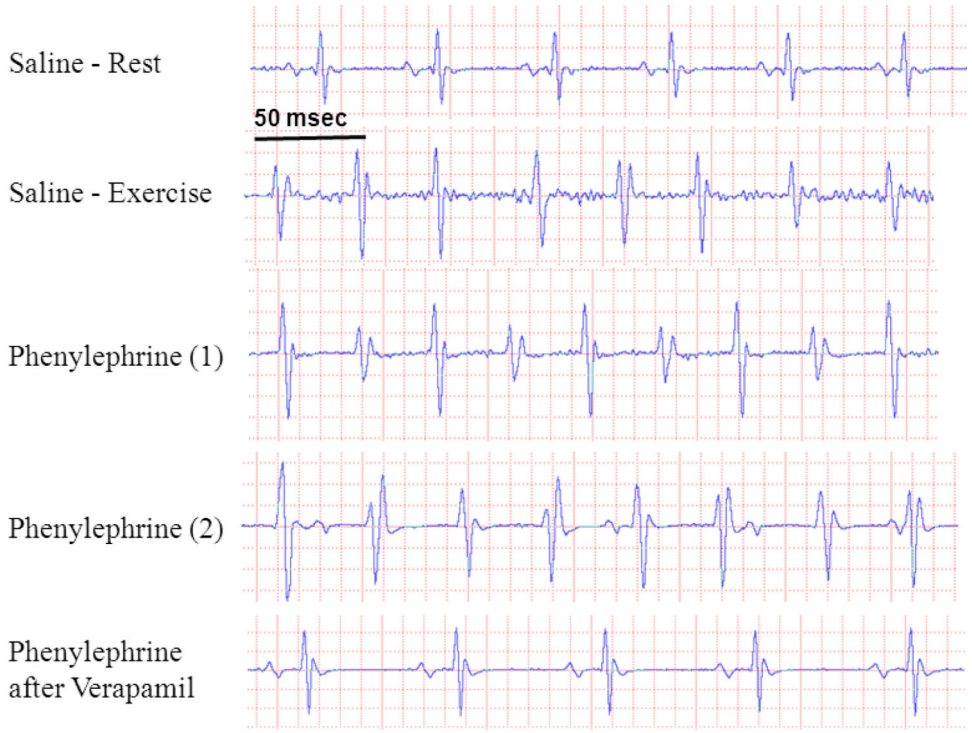

Figure 2.

Representative ECG traces from a 16 week old CASQ2 $^{\Delta/\Delta}$ mouse showing normal sinus rhythm at baseline but polymorphic VT could be evoked by exercise. Ventricular arrhythmia appeared after injection of phenylephrine: trace (1) shows an example of bidirectional VT and (2) shows a polymorphic VT with a prominent atrio-ventricular dissociation. There was no arrhythmia when phenylephrine was administered after pretreatment with verapamil. n=3.

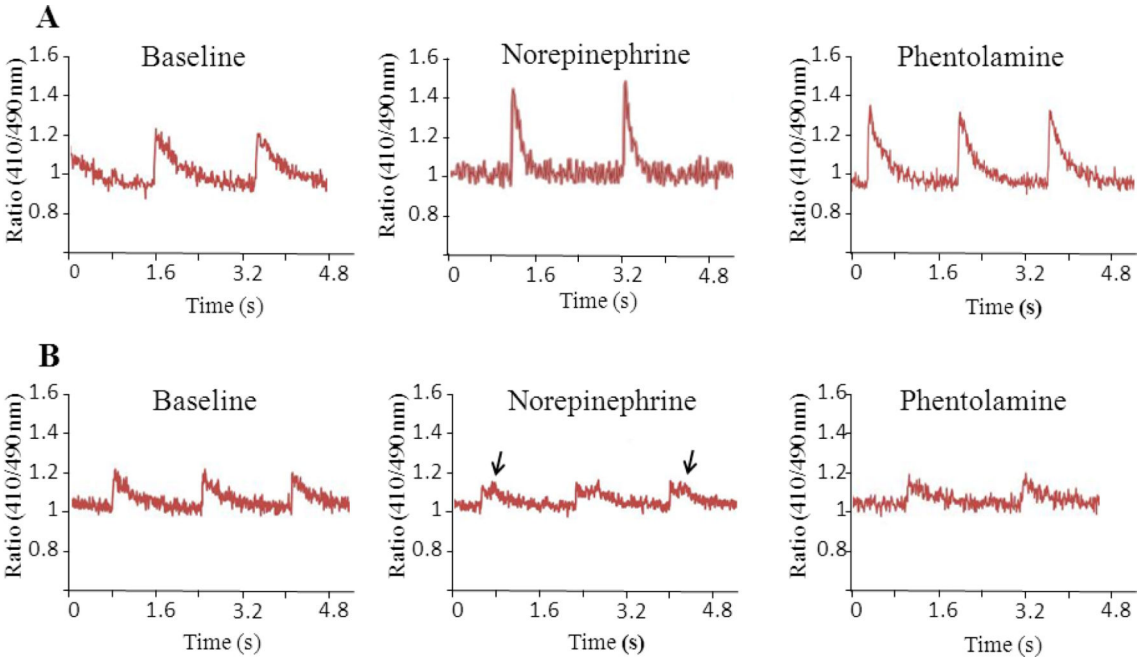

Figure 3.

Calcium transients in cardiomyocytes isolated from wild type (A) and CASQ2$^{\Delta/\Delta}$ (B) mice. Representative traces are shown on baseline, after exposure to norepinephrine and then after adding phentolamine. Note the repetitive abnormal calcium release waves in CASQ2$^{\Delta/\Delta}$ cells exposed to norepinephrine (arrows) which were seen in 4/12 cells studied and disappeared after adding phentolamine (mean±SD, n=3-5 mice/group).

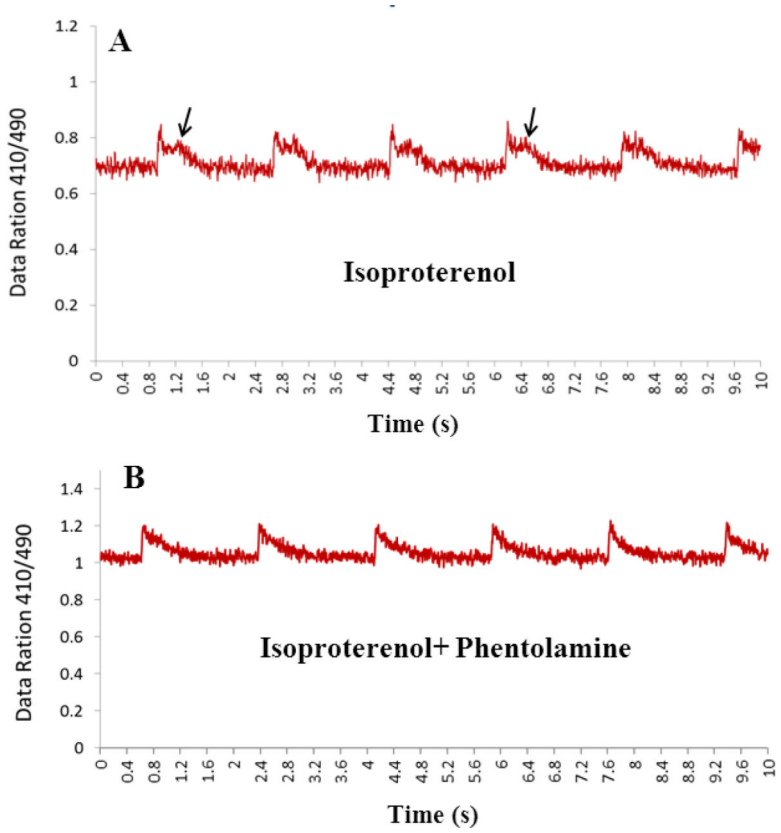

Figure 4.

Calcium transient measurement in isolated CASQ2$^{\Delta/\Delta}$ cardiomyocytes. agonist isoproterenol induces abnormal calcium release waves in 6/6 cells (n=2 mice), which were abolished by adding an blocker phentolamine (B).

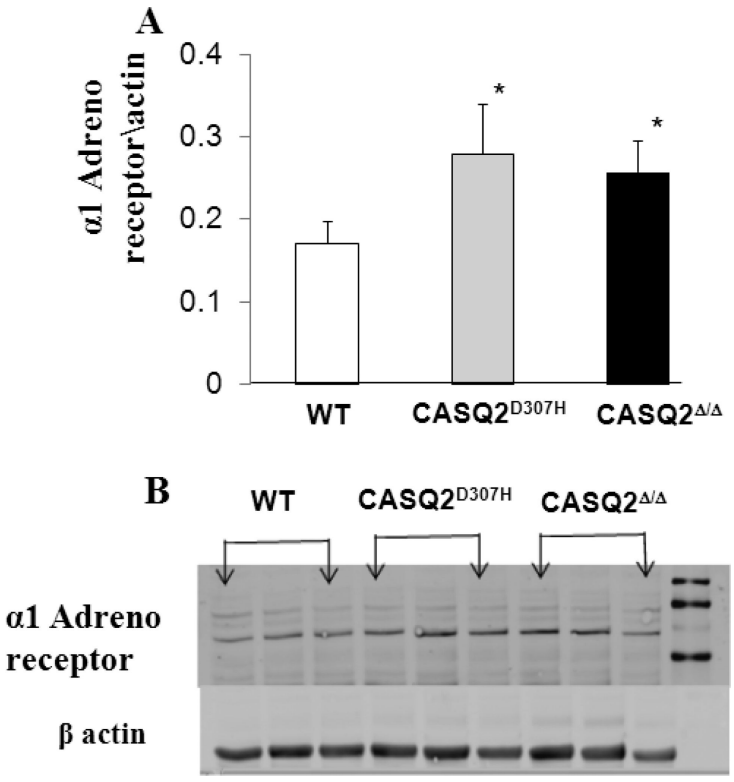

Figure 5.

Western blot for a1adreno-receptor protein in heart homogenates. A. Summary of densitometry results corrected to β actin expression; Y axis – arbitrary units, p*<0.05, n=3/group. B. Blot image.

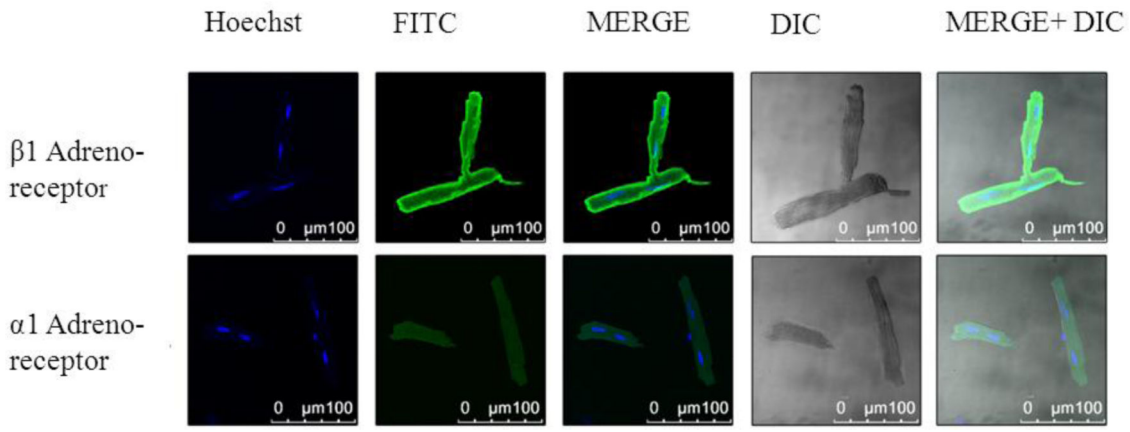

Figure 6.

Confocal microscopy in an isolated CASQ2$^{\Delta/\Delta}$ cardiomyocyte. Cells were stained for either 1adreno-receptor (upper panels) or for 1 adreno-receptor (lower panel).Abbreviations: Hoechst, nucleus staining; FITC, green fluorescence staining; MERG, Hoechst+ FITC; DIC-light microscopy; MERG+ DIC, Hoechst+ FITC+ light microscopy.

Tables Table 1 Pharmacological testing in adult CASQ2$^{\triangle/\triangle}$mice

Agent and Dose	Mechanism of Action	No. mice	Sinus Rate eats/min	Rest			Exercise			Epinephrine			mice with VT
				VPC s	NSV T	SV T	VPC s	NSV T	SV T	VPC s	NSV T	SVT	
Saline 0.1 ml	Control	6	657±22	6	6	0	6	6	4	6	6	5	6
Reserpine acute 0.5mg/g	Neurotransmitter depletion	3	648±10	3	2	0	3	3	1	3	3	3	3
Reserpine – 0.5mg/g	Neurotransmitter depletion	3	637± 6	3	2	0	3	3	2			2	3
Propranolol 10mg/g	β1+pβ2 Blocker	6	456±31¶	3	1	0	6	3	0	6	5	0	5
Metoprolol 10mg/g	β1 Blocker	6	449±11¶	2	0	0	6	5	0	0	0	0	5
Phentolamine 50 mg/g	α Blocker	6	510±45 *	0	0	0	1	0	0	0	0	0	0†
Labetalol 20 mg/g	β1+β2+α1 Blocker	7	409± 6¶	1	0	0	1	0	0	0	0	0	0†
Labetalol 10 mg/g	β1+β2+α1 Blocker	6	460±42¶	2	0	0	4	2	0	0	0	0	2*

Abbreviation: VPCs, ventricular premature contractions; NSVT, non-sutained ventricular tachycardia; SVT, sustained ventricular tachycardia; Sinus rate (beats/min); mice with VT, a criterion for disease expression defined as ventricular tachycardia under any condition;

*P<0.05

†P<0.01

¶P<0.005. The sinus rate was determined at rest.

Table 2

Ca2+ transients in isolated cardiomyocytes from adult WT and CASQ2△/△ mice

	Paramete r	Baseline	P value vs. WT	NE	P value vs. Base	PHENTOL	P value vs. Base	NE + PHENTOL	P value vs. Base
Wildtype	Amplitu de (a.u.)	0.081±0.0 65	NA	0.187 ±0.141	0.004	0.105±0.074	NS	0.140±0.120	NS
	Rise rate (a.u.\sec)	1.45±1.54	NA	3.48±3.96	0.028	1.78±1.15	NS	3.95±5.16	NS
	Decay rate (a.u. \sec)	0.45±0.48	NA	1.29±1.17	0.004	0.51±0.44	NS	1.35±1.48	NS
	AUC (a.u./100)	24±23	NA	40±39	0.13	29±24	NS	31±18	NS
CASQ 2 knockout	Amplitu de (a.u.)	0.056±0.034	0.005	0.059±0.026	NS	0.062±0.014	NS	0.055±0/016	NS
	Rise rate (a.u.\sec)	0.84±0.70	0.004	1.20±0.98	NS	0.96±0.43	NS	1.18±0.76	NS
	Decay rate (a.u.\sec)	0.30±0.36	NS	0.38±0.37	NS	0.36±0.10	NS	0.29±0.12	NS
	AUC (a.u./100)	16±10	0.0012	15±8	NS	13±4	NS	14±5	NS

Abbreviations: a.u., arbitrary units; AUC, area under curve; NE, nor-epinephrine; PHENTOL, phentolamine; NA, non-available; NS, non-significant; all values representmean±SD from ≥12 cells obtained from ≥3 animals.