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Research Article

Post-natal heart adaptation in a knock-in mouse model of calsequestrin 2-linked recessive catecholaminergic polymorphic ventricular tachycardia

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ABSTRACT

Cardiac calsequestrin (CASQ2) contributes to intracellular Ca^{2+} homeostasis by virtue of its low-affinity/high-capacity Ca^{2+} binding properties, maintains sarcoplasmic reticulum (SR) architecture and regulates excitation–contraction coupling, especially or exclusively upon β-adrenergic stimulation. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease associated with cardiac arrest in children or young adults. Recessive CPVT variants are due to mutations in the CASQ2 gene. Molecular and ultra-structural properties were studied in hearts of $CASQ2^{R33Q/R33Q}$ and of $CASQ2^{-/-}$ mice from post-natal day 2 to week 8. The drastic reduction of CASQ2-R33Q is an early developmental event and is accompanied by down-regulation of triadin and junctin, and morphological changes of jSR and of SR-transverse-tubule junctions. Although endoplasmic reticulum stress is activated, no signs of either apoptosis or autophagy are detected. The other model of recessive CPVT, the $CASQ2^{-/-}$ mouse, does not display the same adaptive pattern. Expression of CASQ2-R33Q influences molecular and ultra-structural heart development; post-natal, adaptive changes appear capable of ensuring until adulthood a new pathophysiological equilibrium.

Abbreviations: ANF, atrial natriuretic factor; Bax, Bcl-2-associated X protein; Bcl-2, B cell lymphoma-2; CASQ2, cardiac calsequestrin; CASQ2-R33Q, mutant cardiac calsequestrin; CRT, calreticulin; CRU, calcium release unit; CT1, cardiac triadin; EC coupling, excitation–contraction coupling; Egr-1, early growth response-1; Elk-1, E-twenty-six (ETS)-like transcription factor 1; EM, electron microscopy; ERK1/2, extracellular-signal-regulated kinases 1/2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GRP78, glucose-related protein 78; GRP94, glucose-related protein 94; JC, junctin; jSR, junctional SR; KI, knock-in; KO, knock-out; NCX-1, Na^+/Ca^{2+} exchanger; PCs, peripheral couplings; PI3K, hosphatidylinositol 3-kinases; RYR2, ryanodine receptor 2; SE/SD, standard error/standard deviation; SERCA, SR Ca^{2+}-ATPase; SOCE, Store-Operated Calcium Entry; SR/ER, sarco/endoplasmic reticulum; STIM1, stromal interaction molecule 1; TA, triggered activity; TRPC, transient receptor potential channel; T-tubule, transverse-tubule; UPR, unfolded protein response; VT, ventricular tachycardia; WT, wild type

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Introduction

The sarcoplasmic reticulum (SR) is the major organelle responsible for intracellular Ca^{2+} cycling which, in heart, is tightly controlled on a beat-to-beat basis. Ca^{2+} release units (CRUs), intracellular junctions between SR and transverse (T)-tubules, are made up of a quaternary molecular complex containing the ryanodine receptor (RYR2), the SR Ca^{2+} release channel, the anchoring proteins triadin (CT1) and junctin (JC), and calsequestrin (CASQ2). CASQ2 is localized in the lumen of junctional SR (jSR) and plays an important role in regulating myoplasmic Ca^{2+} concentration [1]. CASQ2 not only determines intra-SR Ca^{2+} by virtue of its low-affinity, high-capacity Ca^{2+} binding properties but also seems to maintain SR architecture [2] and regulates excitation–contraction (EC)-coupling, especially or exclusively upon β-adrenergic stimulation [3].

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease associated with cardiac arrest in children or young adults [4]. The diagnosis of CPVT is most commonly established in subjects suddenly and unexpectedly manifesting stress- or emotion-induced ventricular tachycardia (VT) and syncope. The dominant CPVT variant results from inherited abnormalities of intracellular Ca^{2+} regulation caused by mutations in the RYR2 gene [5], whereas the recessive CPVT variant is due to mutations in the CASQ2 gene [6]. The clinical phenotype of the two CPVT variants is virtually identical and both appears to share the same cellular pathogenetic mechanism entailing SR Ca^{2+} overload and spontaneous diastolic SR Ca^{2+} release evoking VT [7–9].

Our laboratories have been studying the functional consequences of the point mutation R33Q located in the first domain of CASQ2. Since the content of CASQ2-R33Q was drastically reduced in myocytes of the adult knock-in (KI) $CASQ2^{R33Q/R33Q}$ mouse, our experimental model of recessive CPVT [9], the post-natal development of KI hearts (from post-natal day 2 to post-natal week 8) was studied in order to assess the ultra-structural and molecular adaptation. The key issues at stake are when CASQ2-R33Q was reduced and when and what type of adaptive responses occurred in heart right after birth. The main findings are (a) morphological changes of jSR and of SR–T tubule junction are evident at post-natal week 2, (b) reduction of CASQ2-R33Q by about 80% is an early post-natal event and is accompanied by a drastic and concurrent reduction of CT1, whereas JC down-regulation is observed only at post-natal week 8, (c) up-regulation of glucose-related protein 78 (GRP78) from post-natal day 2 onward is evidence of endoplasmic reticulum (ER) stress, (d) pro-survival signals are detected from post-natal week 2 onward, as inferred by increase of Bcl-2-associated X protein (Bcl-2), (e) the transcription factor early growth response-1 (Egr-1) and stromal interaction molecule 1 (STIM1), an Egr-1-dependent Ca^{2+}-regulating protein, are up-regulated, (f) only at post-natal week 8, changes are detected in channels associated to SOCE: Orai1 is drastically reduced whereas transient receptor potential channel (TRPC)3 and TRPC6 are up-regulated, and (g) another model of recessive CPVT, the $CASQ2^{-/-}$ mouse, does not display qualitatively and temporally the same molecular adaptive pattern.

Expression of the mutant CASQ2-R33Q and its precocious and persistent removal influence the molecular and ultra-structural heart development; ensuing, multiple, post-natal adaptive responses, including ER stress, are capable of ensuring until adulthood a new, long-lasting pathophysiologic equilibrium to be hampered only under emotional stress or β-adrenergic stimulations.

Material and methods

Antibodies

Source of specific antibodies: CASQ2 from Thermo Scientific; ERK1/2, Orai1, TRPC3 and TRPC6 from Sigma; GRP78, glucose-related protein 94 (GRP94), calreticulin (CRT) from Abcam; Bcl-2, Bcl-2-associated X protein (Bax), Egr-1, total E-twenty-six (ETS)-like transcription factor 1 (Elk-1) and phosphorylated Elk-1, Na^+/Ca^{2+} exchanger (NCX-1) from SantaCruz; caspases 3, 9, 12, Akt and phosphorylated (T308) Akt, phosphorylated (Thr202/Tyr204) ERK1/2, STIM1 from Cell Signaling; antibodies for CT1 and JC, generous gifts from Dr. Isabelle Marty and Dr. Steven Cala, respectively.

Animal models

Transgenic homozygous KI $CASQ2^{R33Q/R33Q}$, transgenic homozygous knock-out (KO) $CASQ2^{-/-}$ and control C57BL6 wild-type (WT) male mice were previously described [9,10]. All animal experimental protocols were approved by the Animal Care and Use Committee of University of Padova.

Electron microscopy (EM)

Fixed hearts were embedded in an epoxy resin and ultrathin sections were cut, stained, and analyzed as previously described [9].

Protein profile of heart homogenates and quantitative densitometry

Hearts were snap frozen in liquid nitrogen and homogenized in 3% SDS, 1 mM EGTA with protease inhibitors. Quantitative western blotting was carried out on whole heart homogenates from WT, KI and KO mice ($n=4$ for each group). Equal amounts of heart homogenates (30–200 μg) were analyzed by SDS-PAGE [9]. Following transfer to membranes, immunoblots were revealed with the corresponding primary antibodies and secondary antibodies conjugated with either alkaline phosphatase or horseradish peroxidase. In the latter case, visualization was achieved using ECL Western Blotting substrate (Pierce). For quantitative densitometry, intensity of each protein band in blots developed only with alkaline phosphatase was determined by Scion Image software. Protein-signal densities were normalized to the corresponding actin-signal densities within a linear relationship of antigen concentration versus signal density. Data were expressed as mean ± standard error (SE). Comparisons between means of two groups were performed by unpaired two-tales Student's *t*-test. Differences were considered significant at $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.005$.