Cell Calcium 71 (2018) 65–74

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Computational modeling of amylin-induced calcium dysregulation in rat ventricular cardiomyocytes

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ARTICLE INFO

Keywords:
Cardiomyocytes
Amylin Ca^{2+} leak
Ca^{2+} transients
Pre-diabetic rats
Ca^{2+} dysregulation

ABSTRACT

Hyperamylinemia is a condition that accompanies obesity and precedes type II diabetes, and it is characterized by above-normal blood levels of amylin, the pancreas-derived peptide. Human amylin oligomerizes easily and can deposit in the pancreas [1], brain [2], and heart [3], where they have been associated with calcium dysregulation. In the heart, accumulating evidence suggests that human amylin oligomers form moderately cation-selective [4,5] channels that embed in the cell sarcolemma (SL). The oligomers increase membrane conductance in a concentration-dependent manner [5], which is correlated with elevated cytosolic Ca^{2+}. These findings motivate our core hypothesis that non-selective inward Ca^{2+} conduction afforded by human amylin oligomers increase cytosolic and sarcoplasmic reticulum (SR) Ca^{2+} load, which thereby magnifies intracellular Ca^{2+} transients. Questions remain however regarding the mechanism of amylin-induced Ca^{2+} dysregulation, including whether enhanced SL Ca^{2+} influx is sufficient to elevate cytosolic Ca^{2+} load [6], and if so, how might amplified Ca^{2+} transients perturb Ca^{2+}-dependent cardiac pathways. To investigate these questions, we modified a computational model of cardiomyocytes Ca^{2+} signaling to reflect experimentally-measured changes in SL membrane permeation and decreased sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) function stemming from acute and transgenic human amylin peptide exposure. With this model, we confirmed the hypothesis that increasing SL permeation alone was sufficient to enhance Ca^{2+} transient amplitudes. Our model indicated that amplified cytosolic transients are driven by increased Ca^{2+} loading of the SR and that greater fractional release may contribute to the Ca^{2+}-dependent activation of calmodulin, which could prime the activation of myocyte remodeling pathways. Importantly, elevated Ca^{2+} in the SR and dyadic space collectively drive greater fractional SR Ca^{2+} release for human amylin expressing rats (HIP) and acute amylin-exposed rats (+Amylin) mice, which contributes to the inotropic rise in cytosolic Ca^{2+} transients. These findings suggest that increased membrane permeation induced by oligomeratization of amylin peptide in cell sarcolemma contributes to Ca^{2+} dysregulation in pre-diabetes.

1. Introduction

Amylin, a 3.9 kD peptide produced by the pancreatic β cells [7], is secreted along with insulin into the blood stream [8]. Human amylin is amyloidogenic, that is, at high concentrations it aggregates into amyloid and fibrils. In contrast, the amylin isoform found in rodents is non-amyloidogenic [6]. Secretion of amylin (and insulin) is increased in individuals with insulin resistance, a metabolic abnormality that precedes the onset of type II diabetes. Enhanced amylin secretion leads to accumulation of amylin aggregates in the pancreas [9] and other organs, including the heart [6]. These amylin deposits have been shown to induce diastolic dysfunction [6], hypertrophy, and dilation [10]. While studies

Abbreviations: SL, sarcolemma; SR, saroplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; NCX, Na^+/Ca^{2+} exchanger; EC, excitation–contraction; AP, action potential; LCC, L-type calcium channel; HIP, human amylin transgenic; NFAT, nuclear factor of activated T-cells; HDAC, histone deacetylases; CaM, calmodulin; P188, poloxamer 188; NKA, Na^+/K^+ ATPase; L-type Ca^{2+} channel current (i_{Ca}), L-type Ca^{2+} current; SBMG, Shannon–Bers–Morroti-Grandi; i_{tof}, fast component outward potassium current; i_{Ks}, slowly inactivating delayed rectifier current; i_{ss}, non-inactivating potassium steady-state current; i_{K1}, inward rectifier potassium current; GA, genetic algorithm; V, voltage; SDA, state decomposition analysis; ODE, ordinary differential equations; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; SB, Shannon–Bers; TnC, troponin C; TRPV4, transient receptor potential cation channel subfamily V member 4; βAR, β-adrenergic receptor

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https://doi.org/10.1016/j.ceca.2017.11.006
Received 18 October 2017; Received in revised form 30 November 2017; Accepted 30 November 2017
Available online 08 December 2017
0143-4160/

correlating human amylin oligomerization in tissue with the onset of pathological states typical of diabetic cardiomyopathy [11] are beginning to emerge [3], the molecular mechanisms linking amylin insult with cellular dysfunction remain incompletely understood. Gaining momentum, however, is the notion that amylin oligomers in cardiac tissue may disrupt normal calcium homeostasis [6], stemming from amylin's moderately cation-selective conductance properties [4,5,12]. While this conductance is small relative to predominant sarcolemmal Ca^{2+} currents including the L-type calcium channel (LCC) and Na^+/Ca^{2+} exchanger (NCX), it nevertheless exhibits largely unexplained effects on perturbing intracellular Ca^{2+} signals and recruiting Ca^{2+}-dependent pathways associated with pathological, hypertrophic remodeling [13].

In the healthy heart, the Ca^{2+}-dependent excitation–contraction (EC) coupling cycle begins with a depolarizing action potential (AP) that modulates sarcolemma (SL) Ca^{2+} fluxes, including contributions from LCC and Na^+/Ca^{2+} exchanger (NCX) [14]. Ca^{2+} entry via LCC and NCX triggers [15] sarcoplasmic reticulum (SR) Ca^{2+} release via ryanodine receptors (RyRs), leading to a rapid increase in intracellular Ca^{2+} (Ca^{2+} transient) that ultimately activates and regulates competent myocyte contraction [14]. The cycle completes as SR Ca^{2+} uptake via the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), as well sarcolemmal Ca^{2+} extrusion via NCX and the sarcolemmal Ca^{2+} ATPase, collectively restore diastolic Ca^{2+} levels. Recently, we reported that this process is perturbed in hearts from rats that express human amylin in the pancreas (HIP), as well as in isolated cardiomyocytes acutely exposed to the peptide (+Amylin conditions) [6]. In both cases, measurements of a passive, trans-sarcolemmal Ca^{2+} leak from isolated myocytes were faster relative to control [6], which suggested that amylin oligomers insert into the membrane to facilitate a non-selective Ca^{2+} current that correlated with increased cytosolic Ca^{2+} transients. Furthermore, in HIP rat myocytes SERCA function was impaired, and the hypertrophic remodeling associated with nuclear factor of activated T-cells (NFAT) and histone deacetylase (HDAC) pathways were activated. Both properties are strongly associated with the progression toward heart failure (HF) [13]. In this study, therefore, we seek to clarify whether and through which mechanisms the human amylin-induced sarcolemmal Ca^{2+} leak leads to myocyte Ca^{2+} dysregulation.

Cardiac computational models are routinely used for exploring intracellular mechanisms of Ca^{2+} signaling and their dysregulation in cardiac tissue [16–22]. We extended one such model, the Shannon–Bers (SB) model of ventricular myocyte Ca^{2+} dynamics [23], to unravel the influence of amylin in the HIP phenotype. Specifically, the revised model reflects our experimentally-measured changes in SL membrane Ca^{2+} permeation as well as decreased SERCA function consistent with acutely-exposed myocytes and transgenic human amylin rats [6]. We find that increased a sarcolemmal Ca^{2+} background current ('leak') arising from human amylin oligomerization was sufficient to reproduce enhanced Ca^{2+} transients previously measured in HIP rats [6]. These simulations implicate increased SR loading and fractional SR Ca^{2+} release work in tandem to magnify Ca^{2+} transient amplitude for the amylin phenotypes, which in turn elevates cytosolic Ca^{2+} load. Accompanying enhanced SR Ca^{2+} loading is an increased fractional release, stemming from both SR and dyadic cleft Ca^{2+} content. Finally, we show higher propensities for calmodulin (CaM) activation under conditions of elevated diastolic Ca^{2+}, which we indicate may trigger the CaM-dependent NFAT remodeling pathway. These findings lead to our hypothesized model of amylin-induced Ca^{2+} dysregulation summarized in Fig. 1.

2. Materials and methods

2.1. Experimental animals

$N = 12$ Sprague-Dawley rats were used in this study. All animal experiments were performed conform to the NIH guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at University of Kentucky. Ventricular myocytes were isolated by perfusion with collagenase on a gravity-driven Langendorff apparatus [6].

2.2. Measurements of Ca^{2+} transients and passive, trans-sarcolemmal sarcolemmal Na^+ and Ca^{2+} leaks

Myocytes were plated on laminin-coated coverslips, mounted on the stage of a fluorescence microscope and loaded with either Fluo4-AM (10 μmol/L, for 25 min) for Ca^{2+} transient recordings or Fura2-AM (10 μmol/L, for 25 min) for measurements of sarcolemmal Ca^{2+} leak. Ca^{2+} transients were elicited by stimulation with external electrodes at a frequency of 1 Hz. The passive trans-sarcolemmal Ca^{2+} leak was measured as the initial rate of Ca^{2+} decline upon reducing external Ca^{2+} from 1 to 0 mM. In these experiments, Ca^{2+} fluxes to and from the SR were blocked by pre-treating the cells with 10 μM thapsigargin for 10 min whereas the Na^+/Ca^{2+} exchanger (NCX) and sarcolemmal Ca^{2+}–ATPase were abolished by using 0 Na^+/0 Ca^{2+} solution (Na^+ replaced with Li^+) and adding 20 μM carboxyeosin, respectively. Sarcolemmal Ca^{2+} leak was measured in control cells and in myocytes incubated with human amylin (50 μM for 2 h) in the absence and presence of the membrane sealant poloxamer 188 (P188, 50 μM) or epoxyeicosatrienoic acid (14,15-EET, 5 μM). Data for HIP rats are from Despa et al. [6].

Na^+ influx was measured as the initial rate of the increase in intracellular Na^+ concentration ($[Na^+]_i$) immediately following Na^+/K^+ ATPase (NKA) inhibition with 10 mM ouabain. As described previously [24], $[Na^+]_i$ was measured using the fluorescent indicator SBFI (TefLabs). The SBFI ratio was calibrated at the end of each experiment using divalent-free solutions with 0, 10, or 20 mmol/L of extracellular Na^+ in the presence of 10 μmol/L gramicidin and 100 μmol/L strophanthidin.

2.3. Statistical analysis

Data are expressed as mean ± SEM. Statistical discriminations were performed using (1) 2-tailed unpaired Student *t*-test when comparing 2 groups and 1-way ANOVA when comparing multiple groups. Statistical analysis was done in GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla, CA). $P < 0.05$ was considered significant.

2.4. Simulation and analysis protocols

2.4.1. Summary of SBMG rat Ca^{2+} handling model

To examine the relationship between increased sarcolemmal Ca^{2+} entry and elevated Ca^{2+} transients reported in rats [6], we adapted a rabbit ventricular myocyte model of Ca^{2+} signaling to reflect handling terms specific to rodents. This choice was based on the initial lack of rat-specific Ca^{2+} handling models available in the literature. Recent computational models of rat cardiomyocyte Ca^{2+} handling have been published [25,26], and are in qualitative agreement with our implementation, as we will discuss below. Myocytes from rats and mice have similar rates of Ca^{2+} relaxation via SERCA, Na^+/Ca^{2+} exchanger (NCX), and minor contributors such as sarcolemmal Ca^{2+}-ATPase and mitochondrial Ca^{2+} uptake (92, 8 and 1%, versus 90.3, 9.2 and 0.5%, respectively) [27,28]. Accordingly, mouse-specific parameters and potassium current changes were introduced into the SB rabbit cardiomyocyte Ca^{2+} model [23] according to Morotti et al. [29] (summarized in Supplement). The resulting model is hereafter referred to as the Shannon–Bers–Morroti-Grandi (SBMG) model. Model equations, 'state' names, current names and initial conditions are provided in the Supplement. As noted in [29], four predominant changes in potassium channels were included: (1) the transient outward potassium current expression for rabbits was replaced with fast transient outward potassium current (i_{tof}) for mice, (2) the slowly activating delayed rectifier current was substituted with a slowly inactivating delayed rectifier