Angiotensin receptor blockade improves the net balance of cardiac Ca$^{2+}$ handling-related proteins in sympathetic hyperactivity-induced heart failure

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**ABSTRACT**

**Aims:** The clinical benefits of angiotensin II type 1 (AT1) receptor blockers (ARB) in heart failure (HF) include cardiac anti-remodeling and improved ventricular function. However, the cellular mechanisms underlying the benefits of ARB on ventricular function need to be better clarified. In the present manuscript, we evaluated the effects of AT1 receptor blockade on the net balance of Ca$^{2+}$ handling proteins in hearts of mice lacking α$_{2A}$ and α$_{2C}$ adrenoceptors (α$_{2A}$/α$_{2C}$ARKO), which develop sympathetic hyperactivity (SH) induced-HF.

**Main methods:** A cohort of male wild-type (WT) and congenic α$_{2A}$/α$_{2C}$ARKO mice in a C57BL6/J genetic background (5–7 mo of age) was randomly assigned to receive either placebo or ARB (Losartan, 10 mg/kg for 8wks). Ventricular function (VF) was assessed by echocardiography, and cardiac myocyte width and ventricular fibrosis by a computer-assisted morphometric system. Sarcoplasmic reticulum Ca$^{2+}$/calmodulin-dependent protein kinase II (SERCA2A, phospho-Ser$^{16}$-PLN, phospho-Thr$^{17}$-PLN, phosphatase 1 (PP1), Na$^{+}$–Ca$^{2+}$ exchanger (NCX), Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and phospho-Thr$^{286}$-CaMKII were analyzed by Western blot.

**Key findings:** α$_{2A}$/α$_{2C}$ARKO mice displayed ventricular dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis paralleled by decreased SERCA2 and increased phospho-Thr$^{17}$-PLN, CaMKII, phospho-Thr$^{286}$-CaMKII and NCX levels. ARB induced anti-cardiac remodeling effect and improved VF in α$_{2A}$/α$_{2C}$ARKO associated with increased SERCA2 and phospho-Ser$^{16}$-PLN levels, and SERCA2:NCX ratio. Additionally, ARB decreased phospho-Thr$^{17}$-PLN levels as well as reestablished NCX, CaMKII and phospho-Thr$^{286}$-CaMKII toward WT levels.

**Significance:** Altogether, these data provide new insights on intracellular Ca$^{2+}$ regulatory mechanisms underlying improved ventricular function by ARB therapy in HF.

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**Introduction**

Heart failure (HF) is a common leading cause of mortality worldwide. During HF progression, the heart is in constant peril of damage from pathological stresses, such as neurohumoral over-activation (Bacurau et al., 2009; Barki-Harrington et al., 2003; Brum et al., 2006).

Several lines of evidence suggest that sustained renin angiotensin system (RAS) activation plays deleterious effects in the heart since increased cardiac angiotensin II levels lead to cardiomyocyte hypertrophy and myocardial fibrosis (Dostal, 2000; Ferreira et al., 2008a; Leenen et al., 2001), and further contribute to HF establishment (Palaniyandi et al., 2009),. The benefit of RAS inhibition in HF has been demonstrated in several randomized clinical trials where ARB-treated HF patients displayed reduced morbidity and mortality (Pitt et al., 1995), and showed a wide range of desirable hemodynamic outcomes (Sweet and Rucinska, 1994). Besides the well-known cardiac anti-remodeling effect of ARB therapy, some clinical trials and experimental studies have shown that sustained ARB therapy also improves ventricular function in HF patients (Crozier et al., 1995; Qing and Garcia, 1992). However, the molecular mechanisms related to ARB therapy-mediated improved ventricular function in HF remains elusive.

Considering that cardiac function is strongly coupled with Ca$^{2+}$ transient in the heart, a positive effect of ARB on cardiac Ca$^{2+}$-handling proteins in HF might be a potential molecular mechanism involved in ARB improved ventricular function. The process involved in excitation–contraction coupling in the heart involves Ca$^{2+}$ release from the sarco/endoplasmic reticulum (SR) initiated by membrane depolarization and subsequent Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels. This triggers further SR-Ca$^{2+}$ release channels (Ryanodine receptors, RyR) via Ca$^{2+}$-induced Ca$^{2+}$ release and produces Ca$^{2+}$ sparks, triggering cardiomyocyte contraction. After contraction, sarcoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) and Na$^{+}$–Ca$^{2+}$ exchanger (NCX)
perform the critical function of promoting muscle relaxation by sequestering Ca\textsuperscript{2+} from the sarcoplasm or extruding it outside the cell, respectively.

Over the last decades, several studies have shown that ventricular dysfunction is related to changes in phosphorylation status of sarco/ endoplasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA2) function (Pogwizd et al., 2001), upregulation of Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger (NCX) (Pogwizd and Bers, 2002) and diastolic Ca\textsuperscript{2+} leak via ryanodine receptors (Ai et al., 2005). We have reported that Ca\textsuperscript{2+} handling impairment is associated with ventricular dysfunction and HF progression (Bueno et al., 2010; Medeiros et al., 2008), where both pharmacological (Bartholomeu et al., 2008) and non-pharmacological (Vanzelli et al., 2010) therapies improved cardiac function by minimizing Ca\textsuperscript{2+} transient abnormalities. Therefore, Ca\textsuperscript{2+} handling impairment often comes with the development of HF and should be considered a prime target for potential new therapeutic approaches.

We have previously shown that mice lacking both \(\alpha_{2A}/\alpha_{2C}\)-adrenoceptors (\(\alpha_{2A}/\alpha_{2C}\)-ARKO) display sympathetic hyperactivity-related cardiac dysfunction and pathological ventricular remodeling, which ultimately lead to HF and increased mortality rate at 7 months of age (Brum et al., 2002). Lately, we also demonstrated that cardiac abnormalities observed in \(\alpha_{2A}/\alpha_{2C}\)-ARKO mice were accompanied by over activation of cardiac RAS and impaired intracellular Ca\textsuperscript{2+} handling (Ferreira et al., 2008a; Pereira et al., 2009; Rogil et al., 2007). Thus, these mice provide a good model for better understanding the effects of cardiac RAS activation on Ca\textsuperscript{2+} handling proteins expression profile in HF. Therefore, the present study was undertaken to evaluate the effects of ARB therapy on the net balance of Ca\textsuperscript{2+} handling proteins in a genetic animal model of SH-induced HF. We hypothesized that ARB treatment would reestablish the net balance of Ca\textsuperscript{2+} handling proteins involved in SR Ca\textsuperscript{2+} release and reuptake in HF mice.

**Methods**

**Animals and procedures**

A cohort of male congenic \(\alpha_{2A}/\alpha_{2C}\)-ARKO mice in a C57BL6/J genetic background and their wild-type controls (WT) were studied at 5 and 7 mo of age. At 7 mo of age, \(\alpha_{2A}/\alpha_{2C}\)-ARKO mice present severe cardiac dysfunction associated with exercise intolerance and increased mortality rate (Brum et al., 2002). Mice were maintained in a 12:12-h light–dark cycle and temperature-controlled environment (22 °C) with free access to standard laboratory chow (Nuvital Nutrientes S/A, Curitiba, PR, Brazil) and tap water. This study was performed according to ethical principles in animal research adopted by Brazilian College of Animal Experimentation (www.cobea.org.br). In addition, this study was approved by the University of São Paulo Ethical Committee (CEP#059).

**AT1 receptor blockade treatment**

To test whether increased cardiac RAS would be functionally involved in Ca\textsuperscript{2+} handling of advanced-stage cardiomyopathy, \(\alpha_{2A}/\alpha_{2C}\)-ARKO mice were randomly assigned to receive either saline (placebo) or AT1 blocker (ARB, losartan, 10 mg/kg in drinking water) for 8 wk (from 5 to 7 mo of age). ARB treatment did not change blood pressure, cardiac function, and structure of WT mice (data not shown). Therefore, we used only one control WT group for further comparisons with \(\alpha_{2A}/\alpha_{2C}\)-ARKO groups.

**Cardiovascular measurements**

Heart rate (HR) and blood pressure (BP) were determined noninvasively using a computerized tail-cuff system (BP 2000 Visitech Systems, Apex, NC, USA) described elsewhere (Johns et al., 1996). Mice were acclimatized to the apparatus during daily sessions over 4 days, one week before starting the experimental period.

Noninvasive cardiac function was assessed by two-dimensional guided M-mode echocardiography, in halothane-anesthetized WT and \(\alpha_{2A}/\alpha_{2C}\)-ARKO mice, before and after the experimental period. Briefly, mice were positioned in the supine position with front paws wide open, and an ultrasound transmission gel was applied to the precordium. Transthoracic echocardiography was performed using an Acuson Sequoia model 512 echocardiographer equipped with a 14-MHz linear transducer. Left ventricle systolic function was estimated by fractional shortening (FS) as follows: \(FS (%) = \left[\frac{LVEDD - LVESD}{LVEDD}\right] \times 100\), where, LVEDD means left ventricular end-diastolic diameter, and LVESD means left ventricular end-systolic diameter.

**Graded treadmill exercise test**

Exercise capacity, estimated by total distance run, was evaluated with a graded treadmill exercise protocol as previously described (Ferreira et al., 2010; Ferreira et al., 2007). Briefly, after being adapted to treadmill exercises over 1 wk (10 min each session), mice were placed in the exercise streak and allowed to acclimatize for at least 30 min. Exercise intensity was increased by 3 m/min (6–33 m/min) every 3 min at 0% grade until exhaustion.

**Structural analysis**

All mice were killed and their tissues were harvested. Cardiac chambers were then fixed by immersion in 4% buffered formalin and embedded in paraffin for routine histological processing. Sections (4 μm) were stained with hematoxylin and eosin for examination by light microscopy. Only nucleated cardiac myocytes from areas of transversely cut muscle fibers were included in the analysis. Quantification of left ventricular fibrosis was achieved by Sirius red staining. Cardiac myocyte width and ventricular fibrosis were measured in the left ventricle free wall with a computer-assisted morphometric system (Leica Quantimet 500, Cambridge, UK).

**Immunoblot**

Immunoblots of heart homogenates from 7-mo-old WT and \(\alpha_{2A}/\alpha_{2C}\)-ARKO were performed according to Towbin et al. (Towbin et al., 1979). Briefly, liquid nitrogen frozen tissues were homogenized in a buffer containing 1 mM EDTA, 1 mM EGTA, 2 mM MgCl\(_2\), 5 mM KCl, 25 mM HEPES (pH 7.5), 100 μM PMSF, 2 mM DTT, 1% Triton X-100, and protease inhibitor cocktail (1:100, Sigma-Aldrich; St. Louis, MO USA). Samples were loaded and subjected to SDS-PAGE in polyacrylamide gels (10%). After electrophoresis, proteins were electroblotted to nitrocellulose membrane (Amersham Biosciences; Piscataway, NJ USA). Equal loading of samples (60 μg) and even transfer efficiency were monitored with the use of 0.5% Ponceau S staining of the blotted membrane. The blotted membrane was then incubated in a blocking buffer (5% nonfat dry milk, 10 mM Tris–HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated overnight at 4 °C with mouse monoclonal antibodies to SERCA2 (1:2,500), PLN (1:500), and NCX (1:2,000); obtained from Affinity BioReagents (Golden, CO USA); rabbit polyclonal antibody to protein phosphatase type 1 (PP1) (1:1000) was obtained from Upstate Biotechnology (Lake Placid, NY); phospho-Ser\(_{16}\)-PLN (1:5000) and phospho-Thr\(_{17}\)-PLN (1:5000) were obtained from Badrilla (Leeds, UK); CaMKII and phospho-Thr\(_{286}\)CaMKII were obtained from Cell Signaling Technology (Beverly, MA USA). Binding of the primary antibody was detected with the use of peroxidase-conjugated secondary antibodies (anti-rabbit or antimouse, 1:5000, for 1.5 h at room temperature) and developed using...
enhanced chemiluminescence (Amersham Biosciences Piscataway, NJ USA) detected by autoradiography. Quantification analysis of blots was performed with the use of Scion Image software (Scion based on National Institutes of Health ImageJ, USA). Samples were normalized to relative changes in GAPDH levels and expressed as percentage of control group.

Statistical analysis

Data are presented as means ± SE. For fractional shortening, HR, BP, distance run, body weight and LVESD measurements, comparisons of ARB-treated α2A/α2C ARKO, placebo-treated α2A/α2C ARKO and WT control mice were performed by two-way analysis of variance (ANOVA) for repeated measurements with a post-hoc testing by Duncan. For cardiac structural analysis, lung wet/dry ratio and protein expression levels, comparison among groups were performed by one-way ANOVA with a post-hoc testing by Duncan. Statistical significance was considered achieved when the value of P was <0.05.

Results

Effect of ARB treatment on exercise intolerance, hemodynamic and cardiac structure and function

Similar to our previous findings (Rolim et al., 2007), α2A/α2C ARKO mice displayed exercise intolerance when compared with age-matched WT mice before treatment period (5 mo-old) (Table 1). Of interest, ARB therapy improved exercise capacity in α2A/α2C ARKO mice toward WT mice values (Table 1) whereas placebo-treated α2A/α2C ARKO mice presented pronounced exercise intolerance compared to before treatment period (5 mo-old). Baseline HR was higher in placebo- and ARB-treated α2A/α2C ARKO mice compared to WT mice, while systolic BP remained unchanged. However, ARB-treated α2A/α2C ARKO mice displayed a significant reduction in HR compared to placebo-treated α2A/α2C ARKO group (7 mo-old).

FS was significantly lower in α2A/α2C ARKO mice compared to WT mice before treatment period (5 mo-old). Eight weeks of ARB treatment increased α2A/α2C ARKO FS toward WT levels (Fig. 1A). Regarding of cardiac remodeling, placebo-treated α2A/α2C ARKO mice displayed increased LVESD (Table 2), cardiomyocyte hypertrophy (Fig. 1B) and more than two-fold increase of cardiac collagen content (Fig. 1C) compared to age-matched WT mice. ARB treatment normalized LVESD, cardiomyocyte width (Fig. 1B) and collagen content (Fig. 1C) in α2A/α2C ARKO mice.

Expression of proteins involved in intracellular Ca2+ handling

Once we have previously demonstrated that the expression of Ca2+ handling proteins is impaired in α2A/α2C ARKO mice (Medeiros et al., 2008), here we investigated whether sustained ARB therapy would restore their expression profile in HF.

As expected, SERCA2 levels were significantly reduced in placebo-treated α2A/α2C ARKO mice compared to WT mice, while ARB not only reestablished SERCA2 expression but increased it above WT levels (Fig. 2A–B). In addition, NCX expression was increased in placebo-treated α2A/α2C ARKO mice whereas ARB reduced NCX expression toward WT levels (Fig. 2A,C). As sarcoplasmic Ca2+ content depends upon the Ca2+ reuptake by SERCA2 relative to transsarcolemmal Ca2+ extrusion by NCX, we calculated the SERCA2:NCX ratio for all groups studied and found that ARB significantly increased SERCA2:NCX ratio in α2A/α2C ARKO mice compared to placebo-treated α2A/α2C ARKO mice (Fig. 2A,D).

Since SERCA activity is PLN-regulated, we further investigated whether PLN protein kinase A (PKA)- and CaMKII-phosphorylation sites (Ser16 and Thr17 residues, respectively) were altered in HF. Phospho-Ser16-PLN levels were increased in placebo-treated α2A/α2C ARKO mice compared to WT group (Fig. 3A–B). ARB-treated α2A/α2C ARKO mice displayed a further increase in phospho-Ser16-PLN levels compared with other groups (Fig. 3A–B). Phospho-Thr17-PLN expression was increased in placebo-treated α2A/α2C ARKO mice (Fig. 3A,C) and ARB dramatically reduced those levels toward WT values (Fig. 3A,C). No significant changes were found for PPI protein levels among groups (Fig. 3A,D).

α2A/α2C ARKO mice displayed higher expression of CaMKII (Fig. 4B) than WT mice. In addition, ARB was efficient in reducing CaMKII protein levels toward WT mice values (Fig. 4A,B). Phospho-CaMKII expression was also increased in α2A/α2C ARKO mice whereas ARB treatment decreased it to WT levels (P=0.08) (Fig. 4A,C).

Discussion

Heart failure is a progressive disorder that involves decreased cardiac output associated with neurohumoral activation (Goldsmith, 2004). It has been demonstrated that RAS and sympathetic nervous system over activation exert a direct deleterious effect on the heart, ultimately leading to cardiac dysfunction and pathological ventricular remodeling (Bacurau et al., 2009; Brum et al., 2002; Oliveira et al., 2009). In order to counteract the aforementioned effects, pharmacological and non-pharmacological therapies, such as β-adrenergic receptor blocker, AT1-receptor antagonist and exercise training have been developed (Mudd and Kass, 2008). In the present investigation, we aimed to evaluate the effects of ARB therapy on the net balance of Ca2+ handling proteins in a genetic animal model of sympathetic hyperactivity-induced HF.

The novel finding of the present study is that, besides its effect on cardiac remodeling, ARB treatment in α2A/α2C ARKO mice increased cardiac expression of SERCA2 and reduced NCX levels toward WT values. In addition, ARB-treated α2A/α2C ARKO mice displayed an increased phospho-Ser16-PLN expression while decreased phospho-Thr17-PLN, CaMKII and phospho-Thr165 CaMKII protein levels compared to placebo-treated α2A/α2C ARKO mice. These outcomes were accompanied by improved ventricular function, represented by

Table 1

| Physiological parameters in wild type and α2A/α2C ARKO mice. |
|---------------------------------|----------------|----------------|----------------|
|                                | Pre-treatment  | Post-treatment |
|                                 | WT ARKO Placebo ARKO ARB | WT ARKO Placebo ARKO ARB |
| Body weight, g                 | 29.9 ± 0.4 | 28.0 ± 0.5 | 28.8 ± 0.3 | 30.9 ± 0.4 | 28.7 ± 0.5 | 28.2 ± 0.6 |
| Distance run, m                | 408 ± 11 | 310 ± 14 | 285 ± 7 | 366 ± 19 | 254 ± 24 | 341 ± 18 |
| Heart rate, bpm                | 590 ± 12 | 650 ± 12 | 651 ± 8 | 591 ± 14 | 717 ± 7 | 663 ± 15 |
| Blood pressure, mm Hg          | 108 ± 3 | 114 ± 2 | 112 ± 2 | 110 ± 3 | 112 ± 2 | 108 ± 3 |
| Lung wet/dry ratio             | – | – | – | 4.6 ± 0.1 | 7.6 ± 0.4 | 5.3 ± 0.4 |

Body weight, distance run, heart rate and blood pressure before and after 8 weeks of ARB treatment. Lung wet/dry ratio was assessed only after 8 weeks of ARB treatment. Values are presented as means ± SEM. and P<0.05 vs. pre-treatment; *P<0.05 vs. WT mice; †P<0.05 vs. placebo-treated ARKO mice.
increased fractional shortening, and a cardiac anti-remodeling effect, as depicted by decreased cardiomyocyte width and diminished collagen content (Fig. 1).

Over the past decades, it has been demonstrated that human failing hearts display impaired intracellular Ca^{2+} handling due, at least in part, to reduced expression and activity of SERCA2 protein.

Table 2
Echocardiographic measurements in wild type and α2A/α2C ARKO mice.

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<thead>
<tr>
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<th>Pre-treatment</th>
<th>Post-treatment</th>
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<tr>
<td></td>
<td>WT</td>
<td>ARKO Placebo</td>
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<tr>
<td>LVESD, mm</td>
<td>3.01 ± 0.07</td>
<td>3.33 ± 0.03*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.88 ± 0.08</td>
<td>3.91 ± 0.04</td>
</tr>
<tr>
<td>IVSs, mm</td>
<td>0.92 ± 0.03</td>
<td>0.77 ± 0.04*</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.66 ± 0.02</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>0.93 ± 0.03</td>
<td>0.77 ± 0.03*</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.66 ± 0.02</td>
<td>0.60 ± 0.02*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>466 ± 7</td>
<td>446 ± 14</td>
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Left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), interventricular septum thickness at systole (IVSs), interventricular septum thickness at diastole (IVSd), left ventricular posterior wall thickness at systole (LVPWs), left ventricular posterior wall at diastole (LVPWd) and heart rate before and after 8 weeks of ARB treatment. Values are presented as means ± SEM. *P < 0.05 vs. WT mice; † vs. placebo-treated ARKO mice. # P = 0.07 vs. WT mice; ‡ vs. placebo-treated ARKO mice.
(Meyer et al., 1995; Schwinger et al., 1999), a Ca\(^{2+}\) ATPase pump responsible for calcium reuptake to SR. In fact, SERCA2 dysfunction results in elevated cytoplasmic Ca\(^{2+}\) concentration and further diastolic dysfunction. Our previous studies have shown that either β\(_{-}\)-adrenergic receptor blocker or exercise training significantly increased cardiac SERCA2 protein levels in HF mice, which was accompanied by improved intracellular Ca\(^{2+}\) transient and cardiac function (Bartholomeu et al., 2008; Rolim et al., 2007). Interestingly, exercise training also decreased cardiac RAS activation in heart failure mice (Pereira et al., 2009), which could have contributed to improved Ca\(^{2+}\) handling and cardiac function in exercised HF mice. Here we showed that sustained ARB therapy also restored SERCA2 expression and improved cardiac function in a HF model that displays cardiac RAS over activation. These data strengthen the contribution of RAS inhibition to cardiac function re-establishment by regulating intracellular Ca\(^{2+}\) handling.

Several studies have shown that sympathetic hyperactivity leads to downregulation of β\(_{1}\)-adrenoceptor (AR) and uncoupling of β\(_{2}\)-AR, which is accompanied by a desensitization of PKA and increased CaMKII signaling pathways (Palermo et al., 1996; Pogwizd et al., 2001). Moreover, shift from PKA to CaMKII pathway is associated with cardiac dysfunction and pathological ventricular remodeling (Schaper et al., 1991).

In fact, a well-conducted study showed a direct cause–effect relationship between CaMKII activity and impaired cardiac function (Zhang et al., 2005). In our study, we observed an increased phosphorylation of PLN at both Ser\(^{16}\) and Thr\(^{17}\), which is important to highlight that sustained ARB treatment differentially affects the phosphorylation of PLN at Ser\(^{16}\) or Thr\(^{17}\). ARB treatment further increased phosphorylation of PLN at Ser\(^{16}\) (main target of PKA) and decreased phosphorylation of PLN at Thr\(^{17}\) (main target of CAMKII). The increased phosphorylation of PLB at Ser\(^{18}\) by ARB treatment would favor the signaling switch towards a predominant PKA pathway that together with increased SERCA 2 expression take part of for ARB treatment-related mechanisms underlying improved ventricular function in HF.

The increased protein levels of CaMKII and its activated form, phospho-Thr\(^{286}\)-CaMKII, paralleled by increased expression of phospho-Thr\(^{17}\)-PLN levels in placebo-treated α\(_{2A}/\alpha_{2C}\)ARKO mice reveal that cardiac CaMKII pathway is over activated. Of interest, ARB treatment not only decreased CaMKII expression but also reduced phospho-Thr\(^{17}\)-PLN protein levels and tended to decrease phospho-Thr\(^{286}\)-CaMKII.
in α2A/α2CKO mice. These data suggest that improved cardiac function associated with sustained ARB treatment may be also due to the diminished CAMKII pathway activation. We have recently reported that CAMKII pathway is hyper-activated in sympathetic hyperactivity-induced HF, and β-blocker therapy reduced its activation and re-established cardiac function (Bartholomeu et al., 2008; Oliveira et al., 2009). Since CAMKII is a well known substrate of angiotensin II receptor-activated kinases (i.e. PKC and MAPK), it is reasonable to speculate that sustained ARB treatment drastically reduces the phosphorylation of downstream CAMKII targets.

Besides impaired cardiac contractile properties, HF is characterized by progressive myocardial remodeling associated with cardiomyocyte loss and ventricular fibrosis. In fact, we observed cardiomyocyte hypertrophy associated with increased collagen volume fraction in α2A/α2CKO mice. Sustained ARB treatment partially decreased cardiomyocyte cross-sectional diameter and ventricular fibrosis. However, the mechanisms by which ARB treatment reverses cardiac structural abnormalities in α2A/α2CKO mice were not addressed in the present study. In addition, the question of whether impaired Ca2+ handling and cardiomyocyte hypertrophy are isolated or interdependent phenomena is beyond the scope of the present study, but it is undoubtedly an interesting topic for future investigations. The cellular mechanisms underlying the benefits of sustained ARB treatment on ventricular function and remodeling in HF may be related to inactivation of selective transmembrane G-protein-coupled receptors-mediated kinases. Angiotensin II receptor-mediated protein kinase C (PKC) activation has been related to decreased myocyte contractility and pathological hypertrophy in HF, whereas sustained Angiotensin II receptor blockade abrogated the deleterious effects of PKC activation in HF (Inagaki et al., 2002). Furthermore, pharmacological inhibition of PKCβII and PKCε isozymes displayed similar response to ARB treatment in HF animal model (Palaniyandi et al., 2009), resulting in improved calcium transient and better ventricular function (Ferreira et al., 2008b).

Conclusions

The present findings provide, for the first time, evidence that sustained ARB treatment rearranged the network of cardiac Ca2+ handling proteins in sympathetic hyperactivity-induced HF mice. The cardiac structural and functional abnormalities were also counteracted by ARB treatment underscore the role of cardiac RAS activation in this process. Taken together, these results provide new insights on intracellular Ca2+ regulatory mechanisms underlying improved cardiac function by sustained ARB treatment in HF.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

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Fig. 4. ARB therapy reduces CaMKII and phospho-Thr286-CaMKII protein levels in HF. (A) Representative blots of CaMKII, phospho-Thr286-CaMKII and GAPDH, (B) CaMKII and (C) phospho-Thr286-CaMKII protein levels in WT, placebo- and ARB-treated ARKO mice. Note that placebo-treated ARKO mice presented increased CaMKII expression and ARB treatment was able to restore those levels to WT values. In addition, ARB-treated ARKO mice presented a trend toward a decrease in values for phospho-Thr286-CaMKII protein levels vs. placebo-treated ARKO mice. Data are presented as means±SE. *P<0.05 vs. WT.


References


