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Inhibition of connexin 43 in cardiac muscle during intense physical exercise

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Endurance training is accompanied by important adaptations in both cardiovascular and autonomic nervous systems. Previous works have shown that the main component of gap junctions in the ventricular myocardium (connexin 43 (Cx43) can be regulated by adrenergic stimulus. On the other hand, training raises vagal and decreases sympathetic tone, while augmenting myocardial sensitivity to sympathetic stimulation during exercise. We therefore evaluated the regulation of Cx43 expression by sympathetic tone during exercise in trained and sedentary mice. Training induced an increase in the protein level of Cx43 by 45–70% under resting conditions. The expression of Cx43 was inhibited in trained but not in untrained mice in response to a 60 min exercise bout. Normal basal expression was restored after 60 min of resting. Cx43 reached a minimum that was not different from basal expression in untrained mice. In accordance, electrocardiography and action potential analysis did not reveal major electrophysiological implications for the drop in Cx43 abundance in trained-exercise mice. We prevented Cx43 inhibition using propranolol, and observed increased basal mRNA levels of β-adrenergic receptors without significant changes in the ratio β_1 to β_2. In conclusion, we showed that Cx43 expression is transiently inhibited by β-adrenergic stimulus in trained mice during acute exercise.

Physical activity promotes modifications in cardiac function. Sporadic exercise leads to an acute cardiovascular response with short-term effects while regular exercise induces remodeling in the architecture of the heart and the vasculature (Kavazis, 2009; Spence et al., 2011). As a result of this remodeling, trained subjects differ from sedentary individuals not only under resting conditions but also in their acute response to exercise load. Moreover, in trained individuals with an underlying cardiac dysfunction, exercise bouts can trigger life-threatening arrhythmias (Futterman & Myerburg, 1998; Thompson et al., 2007).

Endurance training is accompanied by important adaptations in the autonomic control of the cardiovascular system. Training raises vagal and decreases sympathetic tone (De Angelis et al., 2004; Dickhuth et al., 2004) while augmenting myocardial sensitivity to sympathetic stimulation during exercise (De Angelis et al., 2004).

Alterations in the sympathetic tone have been related to changes in the expression of the gap junctional protein connexin 43 (Cx43). During acute myocardial infarction, increased sympathetic stimulation is responsible for the degradation of myocardial Cx43 (Jiang et al., 2008). Conversely, sympathetic denervation results in increased vascular expression of Cx43 and consequently heightened sensitivity to adrenergic agonists (Slovut et al., 2004).

Gap junctions are responsible for the cell-to-cell electrical coupling required for synchronized cardiac contraction. Since Cx43 is the main component of these channels in the ventricular myocardium (Saffitz et al., 2000), downregulation of its expression can decrease conduction velocity and result in the onset of arrhythmic events (Danik et al., 2004). It has been observed that treadmill trained mice present a higher level of expression of Cx43 in the heart under resting conditions (Bellafiore et al., 2007). However, there is a lack of information regarding the regulation of Cx43 in response to acute exercise.

In the present work, we used a murine model of endurance training to evaluate the role of the sympathetic nervous system during exercise in the regulation of ventricular Cx43 expression.

Materials and methods

Animals

Three-month-old male BALB/c mice were used in the experiments. The animals were housed in a climate-controlled room, at 25 °C, on a 12:12-h light-dark cycle, with free access to food and water. Animals were cared for according to the "Revised Guide for

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the Care and Use of Laboratory Animals" (NIH GUIDE, Volume 25, Number 28, August 16, 1996).

Training protocol

At 3 months of age, the mice were randomly assigned to either an 8 weeks exercise training period or an equivalent sedentary period. The trained mice (TM) group was trained 5 days per week. Daily exercise consisted in two swimming sessions separated by 2 h of resting. The temperature and depth of the water was maintained constant at 30 °C and 15 cm, respectively. Mice swam in groups of 15–30 (in a 900 cm^2 area), which prevented floating. Mice began training for 10 min the first day, after which the time was increased by 10 min/day until 2 h/day was reached.

Ventricular weight to body weight ratio was used as an indicator of cardiac hypertrophy. The effect of the training protocol on the protein basal level of Cx43 and Cx40 was evaluated by Western blot (see below). Basal expression of β1-adrenergic receptor (β1AR) and β2-adrenergic receptor (β2AR) at the mRNA level was assessed by reverse-transcription polymerase chain reaction (RT-PCR; see below).

Experimental design

Cx43 expression in response to acute exercise

TM ($n = 38$) and UM ($n = 17$) were divided into two groups, the control group that was evaluated under resting conditions (TM $n = 23$ and UM $n = 11$) and the exercised group that was subjected to a bout of 60 min (TM $n = 17$ and UM $n = 6$). The animals were sacrificed immediately after either exercise or resting and ventricular samples were obtained. Cx43 protein level was determined by Western Blot (see below).

Regulation of Cx43 expression during exercise

We tested the effects of swimming for different times on the cardiac expression of Cx43. We determined ventricular Cx43 expression at the protein level by Western blot and at the mRNA level by RT-PCR (see below).

TM ($n = 24$) and UM ($n = 24$) were divided into three groups, a control group that did not swim (TM $n = 8$, UM $n = 8$), a group that swam 15 min (TM $n = 8$, UM $n = 8$), and a group that swam 60 min (TM $n = 8$, UM $n = 8$), before sacrifice and tissue harvest.

Recovery of Cx43 expression

Twenty-nine TM were divided into four groups: a control group (control) that did not swim ($n = 10$), a group that swam 120 min ($n = 19$) and did not recover (0 min, $n = 9$), another that recovered 15 min (15 min, $n = 5$), and finally a group that recovered for 60 min (60 min, $n = 5$) before sacrifice and tissue harvest. We exercised mice for 120 min instead of 60 min to procure a steady state starting point for the return to resting state of Cx43 expression level after exercise. This experiment was not performed with UM because no significant changes in Cx43 protein expression were observed in this group under any conditions.

Effect of propranolol on Cx43 regulation during intense exercise

Twenty-three TM were divided into four groups: not exercised (NE, $n = 6$) mice were not treated with propranolol and did not swim the day of the experiment. Propranolol not exercised mice group (PNE, $n = 6$) was treated with propranolol and did not swim on the day of the experiment. This group served to establish a baseline for the propranolol effect. The exercised mice group (E, $n = 5$) was not treated with propranolol, swam for 60 min, and was sacrificed immediately after the exercise bout. Finally, a mice group was treated with propranolol (PE, $n = 6$), swam for 60 min, and was sacrificed immediately after finishing. This experiment was not performed with UM because no significant changes in Cx43 protein expression were observed in this group under any conditions.

Drug administration

To evaluate the action of the sympathetic nervous system on Cx43 expression, we used propranolol. The control group received an i.v. injection of ethanol (50 μL, 95%), and the propranolol (β1 and β2 adrenergic receptor blocker) group was injected with propranolol (Sigma-Aldrich; St Louis, Missouri, USA; 1 mg/kg, iv) dissolved in ethanol (50 μL, 95%) as previously described (De Angelis et al., 2004). Two injections were administered; the first 2 h before sacrifice, and the second 1 h before sacrifice.

RT-PCR

RT-PCR assay was performed to quantify the expression of Cx43 mRNA. Immediately after sacrifice, ventricles were isolated and washed twice with cold phosphate buffered saline, blotted dry and weighted. Total RNA was isolated from ventricular myocardium using TRIzol reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. Reverse transcription was carried out using random primers and RevertAid M-MuLV Reverse Transcriptase (Fermentas, Vilnius, Lithuania) according to the manufacturer instructions. Primers used for amplification were:

Cx43: 5′- GGAAAGGCGTGAGGGAAGTACCCAAC and 5′-CCGGGTTGTTGAGTGTTACAGCGAAA.

GAPDH: CCAGTATGACTCCACTCACGGCAA-3′ and 5′-ATACTTGGCAGGTTTCTCCAGGCG-3′.

β1AR: 5′- CTCGCCGTCACCTGGGCCAC and 5′-CTGCT CGCGCAGAGCCACGA.

β2AR: 5′- CTGAGAGCGCCTGGGCACCG and 5′-CGTGGT CTGGCGCTCGGCTT Cx43.

Abundance was expressed as the ratio of Cx43 band density/GAPDH band intensity. Measurements were done with Beta 4.0.3 Scion Image Software (Scion Corporation, Frederick, Maryland, USA).

Western blot

Western blotting with cardiac homogenates was performed as described (Garcia-Gras et al., 2006). Briefly, for protein isolation, the ventricles were homogenized in 1 mL of RIPA buffer and the resultant homogenate centrifuged at 4 °C during 15 min at 15 000 *g*. The supernatant was stored at −80 °C for posterior use. The Western blot was performed with 40 μg of protein per sample. We performed 3–5 times each experiment using duplicate samples. The Western blot data was analyzed using the Beta 4.0.3 Scion Image Software (Scion Corporation).

Langendorff-perfused mouse heart

Ten BALB/c mice (five trained, five untrained) hearts were isolated using the following procedure. Mice were injected with 500 U/g of heparin (i.m.) 60 min before heart isolation. Mice were anaesthetized with enflurane (InelithranR, Abott, Italy) and sacrificed by cervical dislocation. Hearts were quickly removed through a thoracotomy and rinsed in a Krebs–Henseleit solution containing (in mM) NaCl 115.0, KCl 5.0, $MgSO_4$ 1.5, $CaCl_2$ 2.0, $NaHCO_3$ 25.0, and glucose 10 equilibrated with a 95% O_2, CO_2 5% gas mixture. The aorta was cannulated in a custom-built perfusion chamber and the heart was retrogradely perfused with oxygenated Krebs–

Henseleit solution at 37 °C at 1–2 mL/min. Atria were carefully dissected. The cannulated hearts in the Langendorff perfusion system where stimulated with a bipolar electrode connected to a WPI Pulsemaster A300 stimulator (WPI, Sarasota, Florida, USA) in the epicardium near of the center of the anterior left ventricle at a basic cycle length of 150 ms, 0.2 ms duration, and 50 V.

Transmural electrocardiograms and transmembrane action potentials

Silver electrodes were placed on the bath flanking the heart, and in the right ventricular cavity for recording of transmural electrograms. Electrocardiograms (ECG) were recorded with an accoupled differential amplifier ECG 100 and stored in a personal computer through a data acquisition system MP100WSW (Biopac Systems, Goleta, California, USA). Action potentials were measured simultaneously with floating microelectrodes connected to a differential WPI F223 electrometer (WPI). Signals were analyzed manually to determine relevant intervals, using custom software written in G language, National Instruments LabVIEW™ (Laboratory Visual Instrumentation Engineering Workbench. Austin, Texas, USA).

Statistical analysis

Results, expressed as mean ± standard error of the mean (SEM), were analyzed by analysis of variance (ANOVA) with Newman–Keuls *a posteriori* and Student's *t*-test when appropriated. Differences were deemed significant at $P < 0.05$. Values throughout the text are presented as mean ± SEM.

Results

Training-induced cardiac remodeling

The 8 weeks swim training protocol resulted in a significant increment in the heart weight/body weight ratio in trained mice when compared to untrained littermates [Fig. 1(a) TM = 5.14 ± 0.08 vs UM = 4.45 ± 0.06, $P < 0.001$]. At the molecular level, cardiac hypertrophy was accompanied by a rise in the protein content of Cx43, the connexin isoform most expressed in the ventricular myocardium [Fig. 1(b) TM = 0.77 ± 0.05 vs UM = 1.31 ± 0.13, $P < 0.01$]. The protein level of the connexin isoform 40 (Cx40), which is less abundant in ventricular cardiomyocytes, and preferentially expressed in vascular endothelium and conductive bundles, was also evaluated in ventricular samples. In this case, there were no significant changes associated to endurance training [Fig. 1(c) TM = 0.80 ± 0.14 vs UM = 1.19 ± 0.19].

Response to acute exercise in trained and sedentary mice

We tested the hypothesis that adaptations induced by endurance training affects the level of Cx43 in response to an exercise bout. We compared Cx43 protein abundance in ventricular samples from trained and sedentary mice, before and after 60 min of swimming.

Acute exercise caused a drop of 55.43% in Cx43 protein level in the trained group (0 min = 1.32 ± 0.11 vs 60 min = 0.58 ± 0.06, $P < 0.01$) and remained stable in sedentary mice (0 min = 0.93 ± 0.08 vs 60 min = 0.92 ± 0.12, P = NS; Fig. 2). Due to the higher basal level of Cx43 in trained subjects there was no significance when comparing sedentary with trained-exercised mice (Fig. 2 0.93 ± 0.08 vs 0.58 ± 0.06, P = NS). This suggests that there should be no electrophysiological implications associated to the drop in Cx43 level after an exercise bout. To confirm our deduction, we conducted transmural ECGs and action potential measurements on isolated perfused hearts of sedentary-exercised and trained-exercised mice. Both groups presented normal ECG patterns [Fig. 3(a)]. We found no significant variation in the QRS interval between sedentary and trained mice after swimming (Table 1). The differences encountered in the action potential between the two groups were mainly those reported as the result of chronic endurance training in mice by others (Zingman et al., 2011). We observed APD

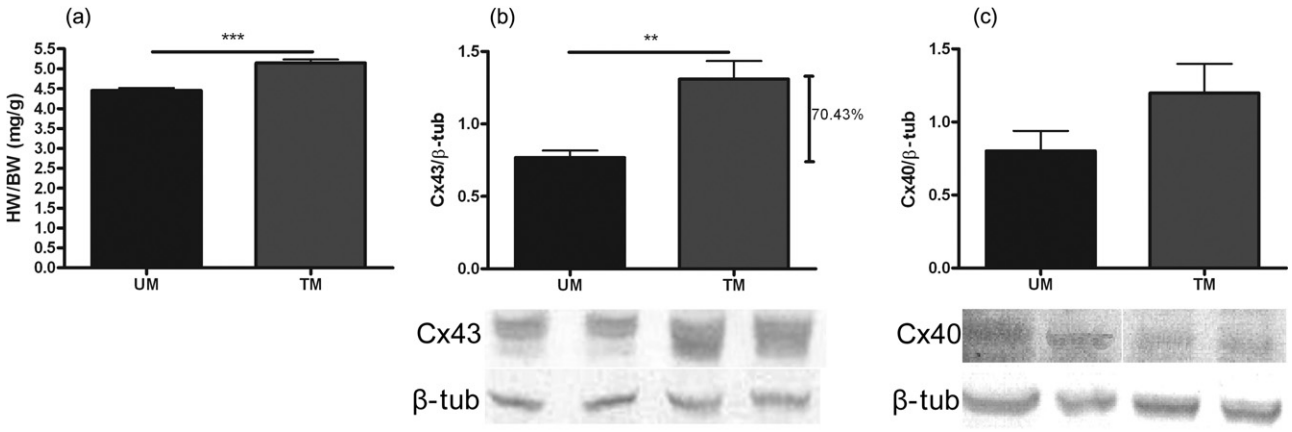

Fig. 1. Heart remodeling in trained mice. (a) Heart weight (HW) to body weight (BW) ratios expressed in mg/g were determinate in control UM ($n = 29$) and TM ($n = 42$) and means ± SEM are plotted. (b) and (c) Inferior panel: representative immunoblot of Cx43 (b) or Cx40 (c) and β-tubulin in UM ($n = 4$) and TM ($n = 4$). Graphics: analysis of Western blots are shown as means ± SEM of Cx43 (b) or Cx40 (c) normalized to the housekeeping β-tubulin; the percentage of rise in Cx43 expression level in TM related to UM is indicated. Means in a and b are significantly different (***$P < 0.001$, **$P < 0.01$).

shortening in the trained-exercise group that also reflected in a reduction in APD90 when compared to sedentary animals [Fig. 3(b) and Table 1].

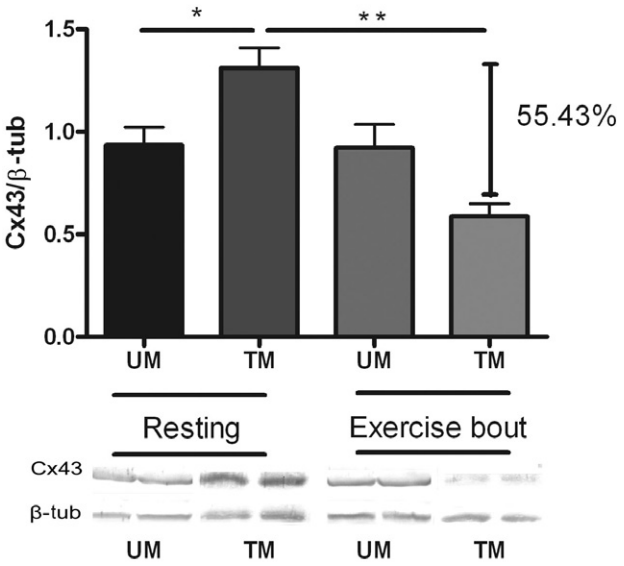

Fig. 2. Cx43 regulation in response to acute exercise in trained and untrained mice. Inferior panel: representative immunoblot of Cx43 and β-tubulin in under resting conditions [UM ($n = 11$) and TM ($n = 23$)] and after an exercise bout of 60 min [UM ($n = 6$) and TM ($n = 17$)]. Graphic: Quantification of Cx43 immunoblots. Cx43 protein level is represented as mean ± SEM related to the housekeeping β-tubulin, $^*P < 0.05$, $^{**}P < 0.01$. The relative fall in Cx43 protein level in TM after exercise respect to TM under resting conditions is indicated.

Kinetics of variation of Cx43 in trained-exercised mice

Cx43 is a short-lived protein, and its abundance is determined by the balance between synthesis and degradation. To gain insight into the kinetics of Cx43 expression level in response to exercise in trained subjects, we measured the RNA and protein levels within a 60 min time frame. In addition, we followed up the recovery to basal levels after an exercise bout of 120 min.

After 15 min of swimming, there was a significant reduction of 53.65% in Cx43 mRNA (1.57 ± 0.17 vs 0.73 ± 0.24 $P < 0.05$) without changes at the protein level (1.37 ± 0.41 vs 1.22 ± 0.49 P = NS). Transcription was further depressed with a bout of 60 min of swimming reaching a reduction of 74.05% (1.57 ± 0.17 vs 0.41 ± 0.12, $P < 0.05$). At this point, protein level was reduced in 48.70% [1.37 ± 0.41 vs 0.71 ± 0.24, $P < 0.05$; Fig. 4(a,b)].

In the recovery phase, transcription showed a marked tendency to increase after 15 min of resting that did not reach significance [Fig. 4(d) 0.24 ± 0.07 vs 0.46 ± 0.12 P = NS]. There was a complete recovery of Cx43 RNA and protein basal levels after 60 min of resting [Fig. 4(d) 1.35 ± 0.46 vs 1.25 ± 0.56 and Fig. 4(c) 0.88 ± 0.17 vs 0.83 ± 0.18, P = NS in both cases].

Effect of sympathetic nervous system stimulation on Cx43 expression

We evaluated the role of the sympathetic stimulation on Cx43 expression by treating trained animals with propranolol before an exercise bout of 60 min.

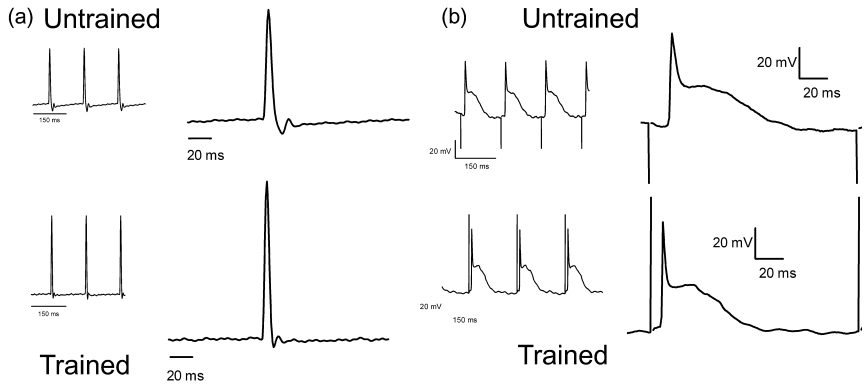

Fig. 3. Cardiac electrophysiology in UM and TM exercise mice. Representative ECG (a) and monophasic action potential (b) recordings performed on isolated Langendorff-perfused hearts of UM ($n = 5$, superior panel) and TM ($n = 5$, inferior panel) after an exercise bout of 60 min.

Table 1. Electrophysiological parameters in UM and TM subject to an exercise bout of 60 min

	RMP (mV)	APD (ms)	APA (mV)	TP (ms)	APD30 (ms)	APD90 (ms)	QRS (ms)
UM	–67.02 ± 0.81	93.09 ± 2.53	64.03 ± 0.77	1.92 ± 0.07	4.34 ± 0.17	63.44 ± 2.16	12.5 ± 0.8
TM	–67.65 ± 0.99	75.35 ± 1.45*	61.82 ± 1.53	1.54 ± 0.07	4.31 ± 0.22	48.60 ± 2.41*	11.1 ± 0.2

The table shows means ± SEM of resting membrane potential (RMP), action potential duration (APD), time to peak (TP), action potential duration at 30% (APD30), action potential duration at 90% (APD90), and QRS interval.
$^*P < 0.05$.

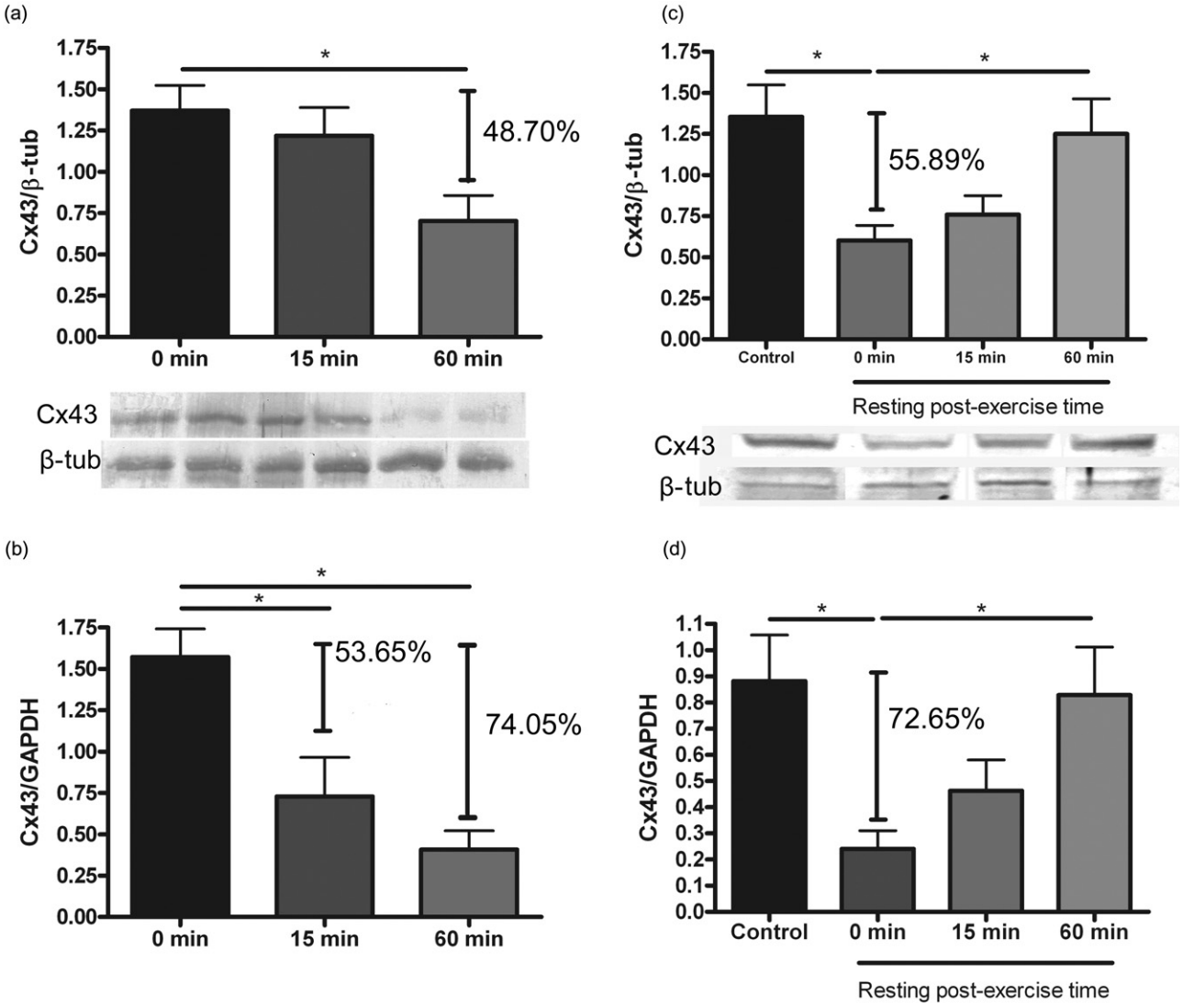

Fig. 4. Kinetic of Cx43 during and after an exercise bout in TM. Cx43 protein (a and c) and mRNA (b and d) levels in TM that exercised 0 ($n = 11$), 15 ($n = 6$), and 60 min ($n = 9$; a and b) or rest after 120 min of swimming for 0 ($n = 9$); 15 ($n = 5$), and 60 min ($n = 5$; c and d). (a and c) Inferior panel: representative immunoblot of Cx43 and β-tubulin. Graphics: quantification of Cx43 immunoblots. Cx43 protein level is represented as mean ± SEM related to the housekeeping β-tubulin (* = $P < 0.05$). (b and d) mRNA levels of Cx43 by RT-PCR semiquantitative analysis normalized to GAPDH expression. The data is shown as mean ± SEM and * = $P < 0.05$. Percentage of decrease is indicated when significant.

The fall in Cx43 expression induced by exercise was completely prevented by propranolol (Fig. 5 0.69 ± 0.07 vs 1.26 ± 0.16, exercised (E) vs resting TM (R), respectively, $P < 0.05$; and 1.26 ± 0.16 vs 1.26 ± 0.13, resting (R) vs propranolol treated-exercised TM (PE), P = NS). In our experimental conditions, treatment with propranolol did not cause significant changes in Cx43 expression under resting conditions [Fig. 4 1.26 ± 0.16 vs 1.14 ± 0.14, resting TM (R) vs resting propranolol treated TM (PR), P = NS].

Since the reduction of Cx43 was specifically observed in trained mice in response to endurance exercise and could be prevented by propranolol, we compared the basal expression of β1 and β2 adrenergic receptors between trained and untrained mice. We observed a significant increase in the expression of both isoforms in trained mice while the ratio β1/β2 remained unchanged [Fig. 6(a) β_1 adrenergic receptor 1.04 ± 0.04 vs 3.08 ± 0.69, and Fig. 6(b) β_2 adrenergic receptor 0.88 ± 0.25 vs 2.02 ± 0.28, UM and TM, respectively, $P < 0.05$ in both cases].

Discussion

In the present work, we have used a murine model to evaluate the effect of acute exercise on the expression of the junctional protein Cx43 in ventricular myocardium of trained and sedentary animals.

The swimming training program we assessed caused a significant increase in mice heart weight to body weight ratio in accordance to previous findings (Kaplan et al., 1994; Evangelista et al., 2003; Yang et al., 2010). At the

molecular level, remodeling of the heart was evidenciated by an increased basal level expression of ventricular Cx43 in trained mice (Bellafiore et al., 2007) without significant changes in Cx40 expression. Yang et al. (2010) using a similar swimming training protocol observed increased levels of both Cx43 and Cx40 expressions. The authors propose that the expression levels of ion channels and connexins are augmented in physiological hypertrophy to preserve cardiomyocyte function. In this sense, the upregulation of gene expression upon training would be concomitant with, and proportional to, increased cardiomyocyte size and membrane capacitance to maintain electrical membrane properties. In our work, the reduction in Cx43 expression observed in trained animals during exercise (to a level similar to that of sedentary mice) did not seem to have appreciable electrophysiological implications. Thus, the rise in basal Cx43 expression does not appear to be a specific adaptation to preserve the connection between larger cells. The results we present suggest that Cx43 content in trained animals exceeds the necessary for normal heart conduction under resting conditions. Further studies on conduction velocity and Cx43 functionality should be performed on our model to establish this condition.

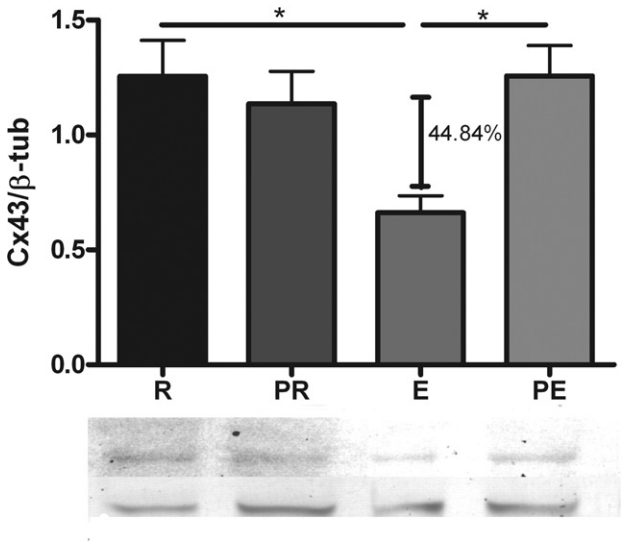

Fig. 5. Effect on Cx43 expression by pharmacologic inhibition of sympathetic nervous system in TM. Inferior panel: representative Western blot of Cx43 and β-tubulin in propranolol treated TM at resting (PR, $n = 5$) or after 60 min exercise bout (PE, $n = 6$), and their respective controls TM at resting (R, $n = 5$) or after exercise bout (E, $n = 5$). Graphic: Quantification of Cx43 immunoblots. Cx43 protein level is represented as mean ± SEM related to the housekeeping β-tubulin (* = $P < 0.05$). The percentage of reduction of Cx43 is indicated when significant.

The half-life of Cx43 has been determined *in vitro* to be 1.5–2 h. It is clasified as a short-lived protein (Laird et al., 1991; Saffitz et al., 2000) and is prone to acute changes in its abundance. The main goal of our study was to demonstrate that Cx43 protein is transiently diminished in trained mice in response to an acute exercise bout of 60 min. Moreover, we show that Cx43 mRNA abundance varies in accordance with Cx43 protein content, suggesting regulation at the transcriptional level. In a preliminary experiment, we intended to assess the effect of 90 min of swimming in sedentary animals. Untrained mice subjected to such an exercise bout often died of extenuation, and therefore, it was an unsuitable group in our experimental model. However, in animals that did survive, we observed a significant drop in Cx43 mRNA and protein levels (data not shown). This could be an indicative of a common mechanism underlying Cx43 regulation in sedentary and trained animals, which is exacerbated by endurance training.

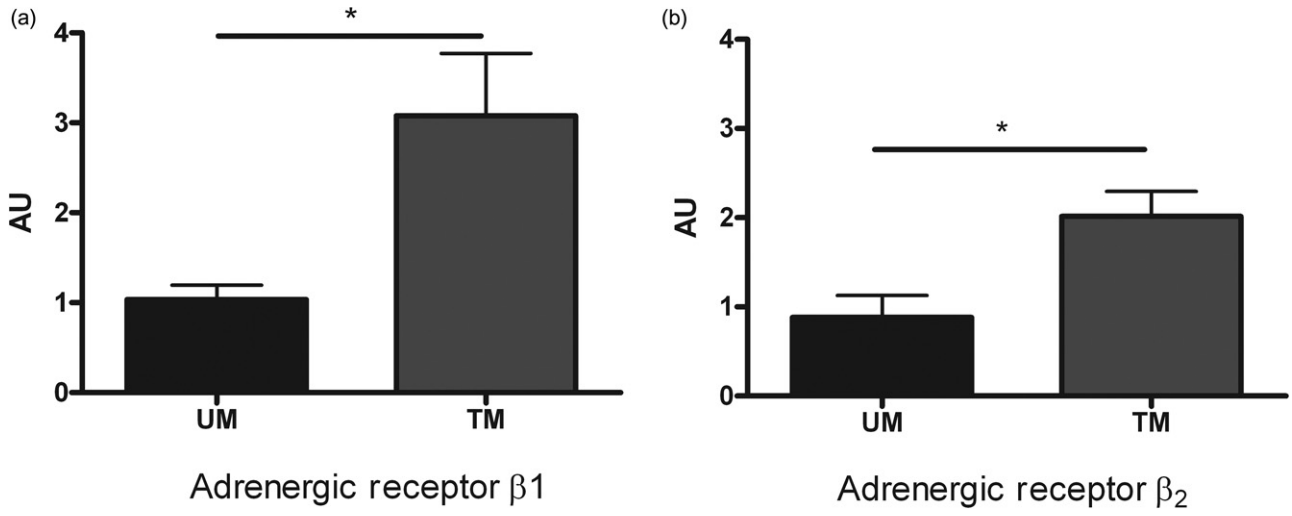

Fig. 6. Expression analysis of adrenergic receptors β_1 and β_2 in TM. Quantification of the RNAm Adrenergic receptors β_1 and β_2 was perform by RT-qPCR in UM ($n = 5$) and TM ($n = 5$). (a) Result of the analysis of adrenergic receptor β_1. (b) Result of the analysis of adrenergic receptor β_2. The data is presented as mean ± SEM of the receptor normalized to GAPDH by the delta-delta ct quantification method. *$P < 0.05$.

It has been reported that the relationship between Cx43 content and conduction velocity is not linear, and that normal cardiac conduction is maintained even after a substantial reduction in Cx43 protein. Danik et al. (2004) generated cardiac-restricted Cx43 conditional knockout mice that gradually decrease the expression of Cx43 in the myocardium after birth. Using this model, the authors have shown that while Cx43 abundance can be a limiting step in conduction velocity, Cx43 expression inhibition is not arrhythmogenic until it reaches a 60% inhibition when compared to age-matched controls. In spite of the acute reduction of Cx43 in trained-exercised animals, we did not observed differences in the ECG pattern or in QRS interval duration when comparing to the sedentary-exercised group. Ideally, ECG should be recorded during exercise to definitively dissociate changes resulting from endurance training rather than from acute exercise, unfortunately during swimming water and movement prevented us from performing a surface ECG. It is important to consider other factors that are potentially arrhythmogenic, which do not involve changes in Cx43, such as changes in refractoriness and heterogeneous distribution of ionic channels (reviewed by Burton & Cobbe, 2001).

A limitation of our study is that we did not evaluate Cx43 at the functional level. In addition to expression changes, Cx43 function and localization can be affected by phosphorylation status by several kinases and phosphatases (Solan & Lampe, 2009). Therefore, we cannot evaluate if such mechanisms are in play in our model. It is also necessary to mention that objections have been raised against the swimming as a model of exercise because as terrestrial animals, swimming causes considerable stress to mice (Masuda et al., 2001). This problem is difficult to circumvent, treadmill running with electrical stimulation suffers a similar limitation and in a voluntary running model, quantification of exercise poses a different but equally confusing challenge (Bernstein, 2003). We have therefore opted for the swimming as it is a widely used and well-established model.

We were able to prevent Cx43 inhibition using the nonspecific beta blocker propranolol, indicating that sympathetic tone is involved in the acute regulation of Cx43 expression during exercise. At the molecular level, we found increased transcriptional levels of β-adrenergic receptors. This result can be interpreted as the chronic postsynaptic adaptation to the diminished central sympathetic tone that has been observed in trained mice (De Angelis et al., 2004). Accordingly, augmented β-adrenergic responsiveness could explain the acute effects on Cx43 in trained-exercise animals.

It has been proposed that the regulation of Cx43 by β-adrenergic stimulus is chronic but not acute (reviewed in Salameh & Dhein, 2011). However, most of the studies that support this hypothesis were performed *in vitro* in neonatal cardiomyocytes where the chronic or subchronic condition is defined as a 24-h incubation with β-adrenergic agonists or AMPc (Salameh et al., 2006) and the acute response was evaluated within minutes (De Mello, 1991). To our knowledge, this work is the first to describe that acute regulation of ventricular Cx43 by β-adrenergic stimulus is differentially induced by exercise *in vivo* in trained-adapted animals.

β_1 and β_2 are the major isoforms of βAR expressed in the mice heart. There is a growing body of experimental evidence suggesting that these two receptors even though they are pharmacologically related have different signaling properties (Aprigliano et al., 1997; Zhou et al., 1997; Xiao et al., 1999, 2006). With regard to endurance training adaptations, the changes in expression and distribution of βAR isoforms are not clearly identified and appear to depend on the experimental setting (Werle et al., 1990; Holycross et al., 2007; Stones et al., 2008). As it was mentioned, we observed increased mRNA basal levels of both isoforms in trained mice. It is of some interest that β_1 to β_2 ratio remained constant since it has been reported that marked overexpression of either β_1 or β_2 isoforms in transgenic mice is associated with the development of pathological heart phenotypes (Zheng et al., 2004). However, the downstream signaling cascades induced by these receptors are out of the scope of our work.

In conclusion, we have described the downregulation of Cx43 expression in trained mice during acute exercise. This feature appears to depend on β-adrenergic sympathetic stimulation.

Perspectives

There is a substantial amount of evidence that support physical activity and exercise training as a mean to improve cardiovascular performance and delay the development of atherosclerosis reducing the incidence of coronary heart disease events (Powell et al., 1987; Fletcher et al., 1996; Lee & Paffenbarger, 2001; Thompson et al., 2003). Nevertheless, exercise can also acutely and transiently increase the risk of acute myocardial infarction and sudden cardiac death in susceptible individuals (reviewed in Thompson et al., 2003 and Ferreira et al., 2010). Epidemiological studies show that the relative risk for sudden death increases during or shortly afterwards of an exercise bout (Sadaniantz & Thompson, 1990). These exercise-associated acute cardiac events generally affect individuals with structural cardiac disease (Ferreira et al., 2010). However, the mechanism underlying the acute effect of exercise in triggering ventricular arrhythmias has not been unraveled. Although some caution must be taken when extrapolating results from murine models to humans, experimental settings with mice still represent a robust approach to evaluate novel hypothesis in the field. We report a mechanism of acute downregulation of Cx43 expression in trained mice that can be examined in murine models of

cardiomyopathies that are associated with sudden death in young athletes (Geisterfer-Lowrance et al., 1996; McConnell et al., 2001; Garcia-Gras et al., 2006; Fabritz et al., 2011).

Key words: sympathetic, gap junction, endurance training, swimming.

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