SHORT COMMUNICATION

Cardiac AT\textsubscript{1} Receptor-Dependent and IGF1 Receptor-Independent Signaling Is Activated by a Single Bout of Resistance Exercise

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Summary

AT\textsubscript{1} receptor (AT1R) blockade prevents physiological cardiac hypertrophy induced by resistance training. Also, our group showed that a single bout of resistance exercise (RE) activates the AKT/mTOR which was also inhibited by AT1R blocker. Here, we investigated whether IGF1-receptor (IGF1-R) and MAPKs were also activated after a single bout of RE. Wistar rats were divided into Sedentary (Sed), Sedentary treated with losartan (Sed+LOS), Exercise (EX), and Exercise treated with losartan (EX+LOS). Cardiac tissue was obtained 5 and 30 min after 4 sets of 12 repetitions of squat exercise (80 % 1RM). We demonstrated that a single bout of RE did not induce IGF1-R tyrosine phosphorylation. ERK1/2 and P38 phosphorylation levels were elevated in the EX 5min and EX 30min groups however, only ERK1/2 was inhibited by losartan treatment (AT1R blocker). Next, we showed that β-arrestin-2 expression increased 28 % in trained animals compared to sedentary group. Altogether, our results demonstrate that AT1R, but not IGF1-R, may exert the hypertrophic cardiac stimulus RE-induced. Also, activation of AKT/mTOR and ERK1/2 pathways may occur through the β-arrestin-dependent pathway.

Key words

AT\textsubscript{1} receptor • Cardiac hypertrophy • β-arrestin • Resistance exercise

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The cardiac hypertrophy (CH) is a well-known response to increased hemodynamic load to the heart and may be induced by different factors such as mechanical stress or neurohumoral factors (Iemitsu \textit{et al.} 2006). CH can be classified as pathological or physiological. The pathological CH occurs in response to situations such as hypertension, valve disease, myocardial infarction or genetic mutations. On the contrary, physiological CH occurs during the normal growth of the heart from embryonic and fetal stages of development until the adult life and also in response to exercise training (Fernandes \textit{et al.} 2015).

It is well established that angiotensin II type 1 receptor (AT1R) plays an important role in the development of pathological CH. However, we have showed increased AT1R expression after exercise training (swimming training) (Oliveira \textit{et al.} 2009), and AT1R blockade has prevented physiological CH induced by exercise training (Barauna \textit{et al.} 2008).

It is generally accepted that activation of G-protein pathway of the AT1R leads to pathological
cardiac remodeling, while G-protein independent pathways, mainly thought β-arrretins, increase cardiac performance, diminish cardiac fibrosis and decreases cardiomyocytes apoptosis (Violin et al. 2014). We have showed that a single bout of resistance exercise (squat exercise) activates the well-known hypertrophic pathway AKT/mTOR pathway thought AT1R in the heart (Melo et al. 2011). However, although the IGF1-R has already been described to be activated by aerobic training (Kemi et al. 2008), it is still unclear its role in response to resistance training.

To further dissect the role of AT1R in the physiological CH exercise-educates, we aimed to verify the AT1R intracellular pathways MAPKs and β-arrestin as well as its crosstalk with IGF1-R in response to resistance training.

Thirty-six male Wistar rats (10-week-old) were randomly divided into six groups (n=6/group): sedentary control (Sed); sedentary control treated with losartan (SED+LOS); exercised and killed after 5 min (EX 5min); exercised and killed after 30 min (EX 30min); exercised treated with losartan and killed after 5 min (EX+LOS 5min) and exercised treated with losartan and killed after 30 min (EX+LOS 30min). Losartan treatment (20 mg.kg⁻¹.day⁻¹) was administered in drinking water instructions (Invitrogen Life Technologies, Strathclyde, UK). β-arrestin-2 mRNA expression was assessed by oligonucleotides primers as follows: 5′-GGACGTTGACATTGA CATTGAGGGGT-3′ and 5′ -GGACGTTGA TGAAGGGGGT-3′. Cyclophilin mRNA expression (5′-TGG CAAGCATGTTGGGTCTTTGGGAG-3′ and 5′ -GGT GGGGT-3′) was measured as internal control. Quantification of the target genes expression was performed with a SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The frozen LVs pieces were homogenized in trizol and RNA was isolated according to the manufacturer’s instructions (Invitrogen Life Technologies, Strathclyde, UK). β-arrestin-2 mRNA expression was assessed by oligonucleotides primers as follows: 5′-GGACGTTGACATTGA CATTGAGGGGT-3′ and 5′ -GGACGTTGACATTGA GGGGT-3′. Cyclophilin mRNA expression (5′-TGG CAAGCATGTTGGGTCTTTGGGAG-3′ and 5′ -GGT GATCTTCCTTGGTCTGTCCATTC-3′) was measured as internal control. Quantification of the target genes expression was performed with a SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The relative expression of the mRNA was performed by real-time PCR in the ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, USA).

Differences between groups (trained vs. sedentary) were assessed using unpaired t-tests. Comparison between groups (with or without losartan treatment vs. sedentary, exercise 5 min or exercise 30 min) were accomplished by two-way ANOVA. Tukey HSD post-hoc test was employed for comparison among mean values, when ANOVA indicated significant changes. P<0.05 was accepted as statistically significant. All results are presented as mean ± standard error of the mean (SEM).

Figure 1 depicts the Western blot results of the MAPK family members, ERK1/2, P38, and JNK proteins in the rat LV. Phosphorylation of ERK1/2 increased in...
the EX 5min (26 %) and EX 30min (59 %) groups, respectively, compared with the sedentary control while losartan inhibited this increase in both groups (Fig. 1A). There was a small, but significant, elevation in P38 phosphorylation in both EX 5min (16 %) and EX 30min (14 %) that was not inhibited by losartan (Fig. 1B). There was no difference in the JNK activation among groups (Fig. 1C). Similarly, Iemitsu et al. (2006) investigated the activation of MAPKs in hearts of rats submitted to aerobic training and also reported increase in P38 and ERK1/2 phosphorylation after 30-min of exercise. The data from our study show that increase in the phosphorylation of ERK1/2 is mediated by AT1R since losartan abrogated this increase. On the other hand, increased P38 phosphorylation was not blocked by AT1R antagonist. Still, it is unclear the linking pathway between AT1R and ERK1/2.

Previous studies showed that the IGF1-R is involved in cardiomyocytes hypertrophy in both elite athletes and trained rats (Scheinowitz et al. 2003). Activation of the IGF1-R is associated with the PI3K/AKT/mTOR pathway when submitted to aerobic training (DeBosch et al. 2006). Since we have already showed AKT/mTOR activation by resistance training (AKT phosphorylation increased 60 % in both the EX 5min and EX 30min groups; mTOR phosphorylation increased 21 % and 65 % in the EX 5min and EX 30min groups, respectively. Both activations were inhibited by losartan (Melo et al. 2011), we next investigated the IGF1-R activation status. Figure 1D shows the IGF1-R phosphorylation level after a single bout of resistance exercise. IGF1-R was immunoprecipitated from LV and subjected to immunoblotting with antiphosphotyrosine antibody. There were no differences in tyrosine phosphorylation levels of IGF1-R. Still, it is also unclear the linking pathway between AT1R and AKT/mTOR.

In summary, the data showed here and previous published by us show that AT1R participates in the activation of both AKT/mTOR and ERK1/2 following a single bout of resistance exercise. AT1R has been

![Fig. 1. MAPKs and IGF1-R activation by resistance training. Effect of a single bout of resistance exercise on the phosphorylation-to-total protein ratio of the MAPK family members and tyrosine phosphorylation of IGF1-R in rat left ventricles (A) ERK1/2 phosphorylation, (B) p38 phosphorylation, (C) JNK phosphorylation, and (D) IGF1-R phosphorylation. Data are reported as mean ± SEM, n=6/group. * P<0.05 vs. Sed group. # P<0.05 vs. EX+LOS 30min group. Sed – Sedentary group without Losartan.](image-url)
shown to regulate both ERK1/2 and AKT in arrestin
signalsomes (Kendall et al. 2014). They observed that
AT1R promoted cell growth and hypertrophy through
β-arrestin-2-mediated mechanisms. To test whether
β-arrestin-2 may participate in the physiological CH
exercise training-induces, rats were subjected to
resistance training for eight weeks. We have observed CH
of 22% in the left ventricular mass/body weight ratio
(Melo et al. 2015) and Figure 2 shows that
β-arrestin-2 gene expression increased by 28%.

Altogether, we have studied the cardiac
intracellular signaling in response to resistance training.
Interestingly, we demonstrated that a single bout of
resistance exercise does not induce IGF1-R tyrosine
phosphorylation, which excludes a possible AT1R and
IGF1-R crosstalk. Also, we suggest that ERK1/2 and
AKT/mTOR activation by resistance training, but not p38
and JNK, may occur by the AT1R β-arrestin-dependent
pathway (Fig. 2). Our results agree with the dual function
of the AT1R both in physiological and pathological
cardiac remodeling which depends on the intracellular
signaling activated. AT1R may have beneficial effects
when it is biased activated to the β-arrestin signaling by
mechanical forces (Rakesh et al. 2010, Barauna et al.
2013), exercise training (Barauna et al. 2008) and biased
ligands (TRV or SII) (Violin et al. 2014). The present
study helps to understand the molecular mechanisms
responsible for mediating the different forms of cardiac
hypertrophy with exercise.

**Conflict of Interest**
There is no conflict of interest.

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