Life Sciences 94 (2014) 24–29

Contents lists available at ScienceDirect

Life Sciences

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

Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats

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ARTICLE INFO

Article history:
Received 3 June 2013
Accepted 21 November 2013

Keywords:
Exercise
Mesenteric
Insulin
Vascular endothelium

ABSTRACT

Aims: We evaluated the mechanisms involved in insulin-induced vasodilatation after acute resistance exercise in healthy rats.
Main methods: Wistar rats were divided into 3 groups: control (CT), electrically stimulated (ES) and resistance exercise (RE). Immediately after acute RE (15 sets with 10 repetitions at 70% of maximal intensity), the animals were sacrificed and rings of mesenteric artery were mounted in an isometric system. After this, concentration–response curves to insulin were performed in control condition and in the presence of LY294002 (PI3K inhibitor), L-NAME (NOS inhibitor), L-NAME + TEA (K^+ channels inhibitor), LY294002 + BQ123 (ET-A antagonist) or ouabain (Na^+/K^+ ATPase inhibitor).
Key findings: Acute RE increased insulin-induced vasorelaxation as compared to control (CT: $R_{max} = 7.3 \pm 0.4\%$ and RE: $R_{max} = 15.8 \pm 0.8\%$; $p < 0.001$). NOS inhibition reduced ($p < 0.001$) this vasorelaxation from both groups (CT: $R_{max} = 2.0 \pm 0.3\%$, and RE: $R_{max} = -1.2 \pm 0.1\%$), while PI3K inhibition abolished the vasorelaxation in CT ($R_{max} = -0.1 \pm 0.3\%$, $p < 0.001$), and caused vasoconstriction in RE ($R_{max} = -6.5 \pm 0.6\%$). That insulin-induced vasoconstriction on PI3K inhibition was abolished ($p < 0.001$) by the ET-A antagonist ($R_{max} = 2.9 \pm 0.4\%$). Additionally, acute RE enhanced ($p < 0.001$) the functional activity of the ouabain-sensitive Na^+/K^+ ATPase activity ($R_{max} = 10.7 \pm 0.4\%$) and of the K^+ channels ($R_{max} = -6.1 \pm 0.5\%$; $p < 0.001$) in the insulin-induced vasorelaxation as compared to CT.
Significance: Such results suggest that acute RE promotes enhanced insulin-induced vasodilatation, which could act as a fine tuning to vascular tone.

Introduction

Several authors have demonstrated the ability of exercise to prevent cardiovascular risk factors, among them endothelial dysfunction (Di Francescomarino et al., 2009; Golbidi and Laher, in press; Green et al., 2004; Zanesco and Antunes, 2007). The literature has demonstrated the ability of both chronic and acute aerobic exercise to improve the insulin signaling pathway involved not only in the glucose metabolism but also in the vascular modulation (Caponi et al., 2013; Pauli et al., 2010; Yang et al., 2006, 2010). In particular, resistance exercise has been also used for improvement of diabetes, hypertension and obesity (Westcott, 2012). Nevertheless, the signaling pathways are not clear.

Hemodynamic effects of insulin occur for two different endothelium-dependent signaling pathways: IR/PI3K/eNOS, responsible for the relaxant effect, and IR/MAPK/ET-1, responsible for the contractile effect (Chaudhuri et al., 2012; Montagnani et al., 2001; Muniyappa and Quon, 2007; Salt, 2013). Thus, the balance between the release of NO and ET-1 plays an important role for the control of vascular tone and blood flow adjustments in response to the exercise (Mather et al., 2001; Muniyappa and Sowers., 2013).

Previous studies have shown that insulin-induced vasorelaxation is enhanced in animals after aerobic exercise. This enhancement is caused by the increase of NO release, associated to K^+ channels-induced hyperpolarization (Ghafouri et al., 2011; Rossi et al., 2005; Yang et al., 2006, 2010). Additionally, Aughey et al. (2007) showed that aerobic exercise can change the activity and expression of skeletal muscle Na^+/K^+-ATPase in humans. In vascular smooth muscle, Garland et al. (2011), Marín and Redondo (1999), and Smith et al. (1997) demonstrated that Na^+/K^+-ATPase activity may be influenced by the endothelium and K^+ channels. However, there are no data in the literature showing the effect of resistance exercise on the insulin-induced relaxation nor the pathways involved in this response.

Previous research has shown the ability of resistance exercise to promote changes in vascular function in rats (Faria Tde et al., 2010; Harris et al., 2010). Interestingly, these changes can be produced in blood vessel far from the skeletal muscle used during the exercise, such as mesenteric or caudal vascular beds (Araújo et al., 2013; Faria Tde et al., 2010). Moreover, it is related in the literature that results obtained in mesenteric vascular bed may have physiological relevance

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0024-3205/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.11.017

for the entire cardiovascular system (Dohi et al., 1994; Subramanian and MacLeod, 2003). These studies evaluated the signaling pathway stimulated by acetylcholine, which is an intracellular calcium-dependent pathway. On the other hand, the signaling pathway stimulated by insulin promotes hemodynamic effects without changes intracellular calcium (Fleming and Busse, 1999).

Therefore, considering it has already been related that resistance exercise may change the metabolic effects of insulin by the IR/PI3K signaling pathway (Krisan et al., 2004; Yaspelkis, 2006), the objective of this study was to evaluate the mechanisms involved in the vasorelaxation induced by insulin after acute resistance exercise in healthy animals.

Material and methods

Animals

Three-month-old male Wistar rats were obtained from the Central Animal Facility of the Federal University of Sergipe. Rats were kept in collective cages (5 animals/cage), temperature-controlled room (22 ± 2 °C) with 12 h light/12 h dark cycle, and received commercial rodent chow (Labina Purina®) and filtered water ad libitum, with free access to food and water. The rats were randomized into four groups: control (CT, n = 20), electrically stimulated (ES, n = 5) and resistance exercise (RE, n = 20). All procedures described in this study are in agreement with the Brazilian Society of Laboratory Animal Science and were approved by the Ethics Committee on Animal Research of the Federal University of Sergipe, Brazil.

Resistance exercise protocol

Animals were exercised following a model described by Tamaki et al. (1992). Rats in the ER and ES groups were wearing a canvas jacket to be able to regulate the twisting and flexion of their torsos and were fixed by a holder in a standing position on their hinder limbs (Tamaki et al., 1992; Barauna et al., 2005; Pinter et al., 2008). Electrical stimulation (20 V, 0.3 s duration, at 3 s intervals) was applied to the tail of the rat through a surface electrode. The animals underwent three days of familiarization, where they were placed in the device in the exercise apparatus starting position and were kept this way for 5 min in order to reduce the stress caused to the animal for the equipment and handling. After the familiarization period, the animals performed the test of a maximum repetition (1RM), which consisted in determining the maximum weight lifted by each rat in the exercise apparatus. The 1RM test is used to assess maximal muscle strength in humans and animals (ACSM, 2009). After 2 days, the animals were subjected to the exercise protocol. The RE group was exercised through 15 sets of 10 repetitions with a 180 s resting period between each set, with the intensity of 70% of 1RM. The animals extended their legs repeatedly, which lifted the weight on the arm of the exercise apparatus. Animals from ES group underwent the same conditions of the animals from RE, but without leg extension movements. The CT group was not subjected to any of these procedures (Fig. 1).

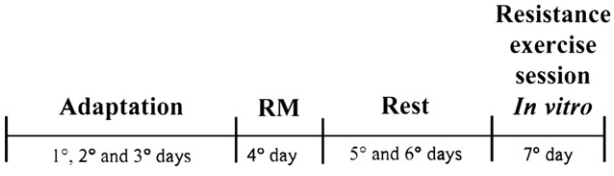

Fig. 1. Diagram showing the resistance exercise protocol.

Vascular reactivity studies

Following animal sacrifice, the superior mesenteric artery was removed, stripped from connective and fatty tissues and sectioned into rings (1–2 mm). Rings were suspended from fine stainless steel hooks, connected to a force transducer (Letica, Model TRI210; Barcelona, Spain) with cotton threads in organ baths containing 10 mL of Tyrode's solution (composition in mM: NaCl 158.3, KCl 4.0, $CaCl_2$ 2.0, $NaHCO_3$ 10.0, $C_6H_{12}O_6$ 5.6, $MgCl_2$ 1.05 and NaH_2 PO_4 0.42). This solution was continually gassed with carbogen (95% O_2 and 5% CO_2) and maintained at 37 °C under a resting tension of 0.75 g for 60 min (stabilization period). During this time, the nutrient solution was changed every 15 min to prevent the interference of metabolites (Altura and Altura, 1970). Isometric tension was recorded through the force transducer (TRI210, Letica, Barcelona, Spain) coupled to an amplifier-recorder (BD-01, AVS, SP, Brazil).

The functionality of the endothelium was assessed by the ability of acetylcholine (ACh, 1 μM) to induce more than 75% relaxation of phenylephrine (Phe, 1 μM)-induced pre-contraction. After that, changes in vascular reactivity were assessed by obtaining concentration–response curves for insulin (10^{-13}–10^{-6} M).

These same curves were obtained after incubation for 30 min of the following inhibitors: L-NAME was used to evaluate the role of NO (inhibitor of nitric oxide synthase; 100 μM); LY294002, to evaluate the role of the PI3K pathway (inhibitor of PI3K; 50 μM); L-NAME + tetraethylammonium (TEA), to evaluate the role of NO and K^+ channels (potassium channel inhibitor; 10 μM); BQ123, to evaluate the role of endothelin-1 (a selective ETA endothelin receptor antagonist; 1 μM); or ouabain, to evaluate the role of Na^+/K^+-ATPase activity in the relaxation (Na^+/K^+-ATPase inhibitor; 100 μM) induced by insulin.

The functional activity of the ouabain-sensitive Na^+/K^+-ATPase was indirectly measured in another set of experiments using the method described by Rossoni et al. (2002). After stabilization in Tyrode's solution with 4.0 mM K^+, the arteries were incubated in a K^+-free medium for 30 min. The vessels were then contracted using phenylephrine and when a plateau was reached, KCl (1–10 mM) was cumulatively added to the bath. To evaluate the functional activity of the ouabain-sensitive Na^+/K^+-ATPase in these responses, the concentration–response curve to K^+ was subsequently determined in vessels pre-incubated for 30 min in a K^+-free medium with ouabain (100 μM).

It is important to note that each ring received only one inhibitor, and the cumulative concentration–response curve for insulin or K^+ was obtained before and after the inhibitor treatment.

Statistical analysis

Values were expressed as the mean ± standard error of the mean (SEM). The maximum response (R_{max} values) was calculated by a non-linear regression analysis of each individual concentration–response curve. AUC were calculated from the individual concentration–response curve plot. Differences of area under the concentration–response curves (dAUC) were expressed between the presence and absence of inhibitors and were expressed as a percentage of AUC of the corresponding control situation. Differences between groups were determined using one- or two-way ANOVA followed by Bonferroni's test or Student's *t* test, as appropriate ($p < 0.05$). Statistical analysis was performed using GraphPad Prism software (San Diego, CA, USA).

Results

Insulin-induced relaxation

Insulin (10^{-13}–10^{-6} M) induced smaller, yet significant, relaxation in a concentration-dependent manner in all groups (Fig. 2). The relaxation induced by insulin was unaltered in the ES group (R_{max} = 7.7 ± 0.5%) as compared to CT group (R_{max} = 7.3 ± 0.4%); however, it was