Peptides 51 (2014) 26–30

Contents lists available at ScienceDirect

Peptides

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

Angiotensin 1–7 improves insulin sensitivity by increasing skeletal muscle glucose uptake in vivo

Omar Echeverría-Rodríguez [a], Leonardo Del Valle-Mondragón [b], Enrique Hong [a,*]

[a] Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Sede Sur, Mexico City, Mexico
[b] Departamento de Farmacología, Instituto Nacional de Cardiología "Ignacio Chávez", Mexico City, Mexico

ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form 14 October 2013
Accepted 15 October 2013
Available online 31 October 2013

Keywords:
Renin–angiotensin system (RAS)
Angiotensin-converting enzyme 2 (ACE2)
Angiotensin 1–7
Mas receptor
Skeletal muscle
Glucose uptake

ABSTRACT

The renin–angiotensin system (RAS) regulates skeletal muscle insulin sensitivity through different mechanisms. The overactivation of the ACE (angiotensin-converting enzyme)/Ang (angiotensin) II/AT_1R (Ang II type 1 receptor) axis has been associated with the development of insulin resistance, whereas the stimulation of the ACE2/Ang 1–7/MasR (Mas receptor) axis improves insulin sensitivity. The in vivo mechanisms by which this axis enhances skeletal muscle insulin sensitivity are scarcely known. In this work, we investigated whether rat soleus muscle expresses the ACE2/Ang 1–7/MasR axis and determined the effect of Ang 1–7 on rat skeletal muscle glucose uptake in vivo. Western blot analysis revealed the expression of ACE2 and MasR, while Ang 1–7 levels were detected in rat soleus muscle by capillary zone electrophoresis. The euglycemic clamp exhibited that Ang 1–7 by itself did not promote glucose transport, but it increased insulin-stimulated glucose disposal in the rat. In a similar manner, captopril (an ACE inhibitor) enhanced insulin-induced glucose uptake and this effect was blocked by the MasR antagonist A-779. Our results show for the first time that rat soleus muscle expresses the ACE2/Ang 1–7/MasR axis of the RAS, and Ang 1–7 improves insulin sensitivity by enhancing insulin-stimulated glucose uptake in rat skeletal muscle in vivo. Thus, endogenous (systemic and/or local) Ang 1–7 could regulate insulin-mediated glucose transport in vivo.

1. Introduction

Skeletal muscle is the predominant site for glucose disposal in the postprandial state, and insulin-stimulated glucose uptake in this tissue represents the most important process for maintaining glucose homeostasis [6]. In insulin resistance states such as obesity, hypertension, and type 2 diabetes, insulin-induced glucose transport is markedly decreased in skeletal muscle, due to an impaired expression and functionality of the insulin signaling pathway [11].

The mechanisms underlying skeletal muscle insulin resistance are multifactorial. Of paramount importance, the RAS regulates skeletal muscle insulin sensitivity through different mechanisms. The overactivation of the classical pathway of RAS, the ACE/Ang II/AT_1R axis, has been associated with the development of insulin resistance in skeletal muscle [11,14]. In contrast, recent studies show that the stimulation of the novel pathway of the RAS, the ACE2/Ang 1–7/MasR axis, acts in the opposite way, i.e. it improves skeletal muscle insulin sensitivity [10,21,25].

The in vivo mechanisms by which the ACE2/Ang 1–7/MasR axis improves skeletal muscle insulin sensitivity have not been extensively characterized, even though it is known that skeletal muscle expresses ACE2 [8] and MasR [18], suggesting that Ang 1–7 could be locally synthesized. At the functional level, the i.v. administration of Ang 1–7 in normal rats stimulates the phosphorylation of the insulin signaling effector Akt (protein kinase B) in skeletal muscle, liver, and adipose tissue (insulin target tissues) [18]. Furthermore, in fructose-fed rats, Ang 1–7 infusion reverts insulin resistance by restoring the decreased activation of the insulin signaling pathway, including insulin receptor, IRS-1 (insulin receptor substrate 1), PI3K (phosphatidylinositide 3-kinase), and Akt, in the insulin target tissues [10]. It has been also observed that Ang 1–7 reduces Ang II-induced insulin resistance by increasing the phosphorylation of Akt in isolated rat skeletal muscle [21]. In addition, Ang 1–7 increases the protein expression of GLUT4 (glucose transporter 4) in both skeletal muscle from ACE2 knockout mice, and in C2CI2 myotubes [25]. These results clearly indicate that Ang 1–7 positively regulates the insulin signaling in skeletal muscle. In support

* Corresponding author at: Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Sede Sur, Czda. de los Tenorios No. 235, Col. Granjas Coapa, Del. Tlalpan, C.P. 14330 México D.F., Mexico. Tel.: +52 55 5483 2864; fax: +52 55 5483 2863.
E-mail addresses: enriquehong@hotmail.com, ehong@cinvestav.mx (E. Hong).

0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.10.022

of this contention, recent reports exhibit that Ang 1–7 stimulates glucose uptake in both 3T3-L1 adipocytes [16] and C2C12 myotubes [25].

Considering the above findings, we hypothesized that Ang 1–7 would improve insulin sensitivity by regulating skeletal muscle glucose disposal in vivo. We aimed to investigate whether rat soleus muscle expresses the ACE2/Ang 1–7/MasR axis, and to determine the effect of Ang 1–7 on glucose uptake in rat skeletal muscle in vivo.

2. Materials and methods

2.1. Animals

Eight weeks-old male Wistar rats (weighing 250–300 g) were used for this study and were provided by our animal facility. The animals were maintained under controlled conditions of light/dark cycles (12/12 h), temperature (22 ± 1 °C), and humidity (50 ± 10%), and were fed with standard laboratory diet and water ad libitum. All experimental procedures were conducted in accordance with our Federal Regulations [19], which follow the Guidelines for Care and Use of Laboratory Animals, and were approved by our Institutional Ethics Committee (CICUAL, Protocol 127-03).

2.2. Collection of tissue

Six rats were killed by decapitation and samples of skeletal muscle (soleus) were collected. The samples were immediately frozen in liquid nitrogen and stored at −70 °C for later analysis.

2.3. Quantification of Ang II and Ang 1–7

Simultaneous determination of both Ang II and Ang 1–7 was performed in soleus muscle by capillary zone electrophoresis coupled with photodiode-array detection (CZE-PDA) [26]. The samples (∼30 mg) were homogenized in a cold mixture of methanol (500 μl) and phosphate buffer (500 μl, pH 7.4, 50 mM), and centrifuged at 13 000 rpm for 15 min at 4 °C. The pellets were discarded and the supernatants (300 μl) were diluted with NaOH (1:1, 0.1 M) and incubated for 3 h at 4 °C. The samples (200 μl) were diluted (1:10) in a cold mixture (1:1) of methanol and perchloric acid (5%, w/v). The pH of the mixture was adjusted to 2.0 ± 0.1 and centrifuged at 13 000 rpm for 15 min at 4 °C. The supernatants (100 μl) were passed through a Sep-Pak Classic C-18 cartridge (Waters Corporation, Milford, MA, USA) and mixed with 10 μl of a solution (1:1:1) of water–methanol–acetic acid (1%, w/v). The samples were filtered with a nitrocellulose membrane (0.22 μm) and analyzed by CZE-PDA (P/ACE^{TM} MDQ, Beckman Coulter, Fullerton, CA, USA).

2.4. Western blot

Forty micrograms of proteins were extracted from rat soleus muscle (∼150 mg of tissue), separated on SDS-PAGE (10%), and transferred on PVDF membranes. The membranes were incubated overnight at 4 °C with the following primary antibodies: monoclonal mouse anti-ACE2 (1:1000 dilution; Millipore, cat. no. MAB5676), polyclonal goat anti-Mas R (1:1000 dilution; Santa Cruz Biotechnology, cat. no. sc-54848), or monoclonal mouse anti-actin (load control; 1:10 000 dilution; Millipore, cat. no. MAB1501R). Then the membranes were incubated 1 h at room temperature with their corresponding secondary antibody: goat anti-Mouse (1:5000 dilution; Jackson ImmunoResearch, cat. no. 115-035-003), rabbit anti-goat (1:5000 dilution; Jackson ImmunoResearch, cat no. 305-035-003), or goat anti-Mouse (1:10 000 dilution). After incubation, the blots were visualized using a chemiluminescence kit (Immobilon Western, Millipore, MA, USA) and exposed to photographic films (Kodak, USA). The bands were quantified by densitometry employing the UVP EpiChemi System with the Labworks 4.5 software. The results are expressed by the relationship protein/actin in units of relative density.

2.5. Glucose uptake

The hyperinsulinemic euglycemic clamp was used to determine the effect of Ang 1–7 on glucose uptake in vivo [17]. After an overnight fast, the rats were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.). One third of each dose was administered when was required. The animals were placed under a heating lamp to ensure euthermia and the trachea was cannulated, and then connected to an air pump (Searle Bioscience; 56 strokes/min; stroke/volume: 20 ml/kg) to maintain continuous ventilation. Polyethylene tubes were placed into: (1) right carotid artery for blood pressure recording (Grass Instrument Co., model 7, Quincy, MA, USA); (2) left and right jugular veins for infusions of insulin (4 mU/kg/min; HUMULIN® R, Eli Lilly Co., IN, USA) and glucose (25%, w/v, variable infusion rate), respectively; (3) left and right femoral veins for infusions of saline (0.02 ml/min), Ang 1–7 (67 ng/kg/min; Sigma–Aldrich Co., St. Louis, MO, USA), Ang 1–7 in the absence (0–70 min) and presence (70–120 min) of insulin, captopril (30 μg/kg/min; donated by TEVA Pharmaceuticals, Mexico), or captopril + A-779 (30 μg/kg/min; Bachem, PA, USA); and (4) left femoral artery to take blood samples.

When the animals presented stable hemodynamic parameters and anesthesia (for 15–30 min), the blood glucose was determined (baseline level) using a glucometer (Accu-Check Active, Roche, Mannheim, Germany). Then rats were subjected to the following protocols (Fig. 1). In the protocol A, they received systemic infusions of saline or Ang 1–7; while the rats of the protocol B received infusions of saline, captopril, or captopril + A-779. After 30 min, blood glucose was measured and insulin infusion was started (for 120 min). In the case of Ang 1–7 per se, insulin was infused at min 70 (Fig. 1A). Blood glucose concentrations were determined every 10 min during 2 h. Glucose solution was infused at a variable rate to maintain baseline blood glucose levels. Glucose infusion rate (mg/kg/min) was calculated to evaluate insulin sensitivity.

2.6. Statistical analysis

Results are expressed as means ± SEM. The euglycemic clamp data were examined employing the two-way ANOVA followed by the Student–Newman–Keuls post hoc test. The effect of Ang 1–7 on the glucose infusion rate in the absence (0–70 min) and presence (70–120 min) of insulin was analyzed using the one-way RM ANOVA. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Skeletal muscle expresses the ACE2/Ang 1–7/Mas axis of the RAS

Western blot analysis revealed the expression of ACE2 and MasR in rat soleus muscle (Fig. 2A and B). In addition, capillary zone electrophoresis allowed the detection of Ang II and Ang 1–7 levels in muscle tissue (Fig. 2C).

3.2. Ang 1–7 increases skeletal muscle glucose uptake in vivo

Acute infusion of Ang 1–7 (67 ng/kg/min) increased the insulin-induced glucose infusion rate in comparison with the saline infusion (Fig. 3A). Ang 1–7 infusion by itself, i.e. in the absence of insulin (0–70 min), did not stimulate glucose disposal (Fig. 3A), that is, the glucose infusion rate was not different to zero