European Journal of Pharmacology 656 (2011) 68–73

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular Pharmacology

Alterations in perivascular adipose tissue structure and function in hypertension

Chao Lu, Li-Ying Su, Robert M.K.W. Lee, Yu-Jing Gao *

Smooth Muscle Research Program, McMaster University, Hamilton, Ontario, Canada
Department of Anesthesia, McMaster University, Hamilton, Ontario, Canada

ARTICLE INFO

Article history:
Received 24 April 2010
Received in revised form 13 December 2010
Accepted 12 January 2011
Available online 28 January 2011

Keywords:
Aorta
Brown adipocyte
Contraction
Hypertension
Perivascular adipose tissue
Spontaneously hypertensive rat
Uncoupling protein-1

ABSTRACT

We studied the structural and the functional alterations of perivascular adipose tissue (PVAT) in hypertension with spontaneously hypertensive rats (SHR). Measured with dual energy X-ray absorptiometry, a smaller body fat mass and a greater lean mass were found in SHR than in Wistar–Kyoto (WKY) rats, while body weight was comparable between them. In the thoracic PVAT, the density and the total number of brown adipocytes were greater in SHR than in WKY rats, while the cross section area of PVAT was similar between them. In functional assessment, four types of vessel preparations (with either intact PVAT or intact endothelium, or with both, or without both) were employed. Vessels with intact PVAT from SHR contracted more to phenylephrine than that from WKY rats, while vessels without PVAT exhibited comparable contractile response to phenylephrine between SHR and WKY rats. Both endothelium-dependent and -independent components of PVAT-associated attenuation of phenylephrine-induced contraction were reduced in SHR as compared with that of WKY rats. Bioassay experiments were carried out to assess the transferable relaxation factor from the PVAT. Transfer of bathing solution incubated with PVAT-intact vessel caused less relaxation in SHR recipients than in WKY rats, and the relaxation response was abolished by D-Ala^7-angiotensin-(1–7), an angiotensin-(1–7) receptor antagonist. In summary, PVAT-associated inhibition of vessel contractile response to agonist was impaired in SHR, and the impairment involved both endothelium-dependent and -independent mechanisms. The functional impairment observed in SHR PVAT may be related to changes in adipocyte composition but not to reduced PVAT mass in SHR.

1. Introduction

Mounting evidence has shown that perivascular adipose tissue (PVAT), which surrounds most of systemic arteries, is a modulator of vascular function (Gollasch and Dubrovska, 2004; Gao, 2007). Relaxation factors produced by PVAT attenuate vasoconstriction to various agonists (norepinephrine, phenylephrine, serotonin, angiotensin II, and U 46619), or induce vasodilation of pre-contracted vessels in several vascular beds and species including human (Soltis and Cassis, 1991; Lohn et al., 2002; Gao et al., 2005a,b; Takemori et al., 2007; Malinowski et al., 2008). PVAT-mediated attenuation of contraction involves both endothelium-dependent and -independent mechanisms as shown in rat aorta (Gao et al., 2007). One of the endothelium-dependent relaxation factors in rat aortic PVAT has been identified as angiotensin-(1–7), which induces relaxation through endothelial NO release (Lee et al., 2009a). The endothelium-independent mechanism involves production of hydrogen peroxide by PVAT and subsequent activation of soluble guanylyl cyclase (Gao et al., 2007).

Further studies in diseased animal models have revealed that altered PVAT function is involved in several pathophysiological conditions. Impaired PVAT-associated inhibition of contraction has been found in the aorta and mesenteric arteries of rats exposed to nicotine *in utero*, and these rats exhibited obesity and elevated blood pressure in their adult life (Gao et al., 2005a, 2008). On the other hand, enhanced PVAT function in attenuating vasoconstriction has been observed in hyperglycemia and diabetic rats (Lee et al., 2009b). In hypertension, an impaired PVAT function has been reported in the mesenteric arteries of spontaneously hypertensive rats (SHR) (Galvez et al., 2006; Galvez-Prieto et al., 2008). However, whether the functional alterations observed in SHR is associated with structural or morphological changes of PVAT and the involvement of the endothelium-dependent and -independent mechanisms in the impairment remain to be identified. Here we carried out a combined structural and functional investigation to define alterations in the PVAT of SHR.

2. Materials and methods

2.1. Animals

Male SHR and WKY rats were obtained from the colonies at the Central Animal Facilities of McMaster University. These colonies were

* Corresponding author at: Department of Anesthesia, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5. Tel.: +1 905 525 9140x22477; fax: +1 905 523 1224.
E-mail address: gaoyu@mcmaster.ca (Y.-J. Gao).

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doi:10.1016/j.ejphar.2011.01.023

originally derived from Charles River and have maintained in our facility for over 30 years. The study conforms with both the Guide for the Care and Use of Laboratory Animals published by the US national Institutes of Health and the Guidelines of the Canadian Council on Animal Care, and was approved by the Animal Research Ethics Board at McMaster University, in accordance with the guidelines of the Canadian Council of Animal Care.

2.2. Blood pressure and in vivo body fat mass measurement

Blood pressure was measured by the tail-cuff method. Total body fat contents were measured with dual energy X-ray absorptiometry under light aesthesia with isoflurane (Mathai et al., 2008).

2.3. Measurement of PVAT cross-section area, and immunohistochemical staining for brown adipocytes with uncoupling protein 1 (UCP 1)

Animals were sacrificed with an overdose of sodium pentobarbital (60 mg/kg, ip) and segments from the middle part of thoracic aorta with intact surrounding tissues were fixed in 10% formaldehyde at 4 °C overnight, washed in water and embedded in paraffin. Cross sections (5 μm) were deparaffinized in xylene, rehydrated and washed in physiological basic salt solution (PBS). PVAT cross-sectional area was measured in hematoxylin/eosin stained sections under light microscopy. UCP 1 was used as a marker to differentiate brown adipocytes from white adipocytes in PVAT (Cinti, 2002) to examine compositional alterations in PVAT between SHR and WKY rats. Endogenous peroxidase activity was quenched in hydrogen peroxide for 10–15 min and then the sections were washed four times in PBS buffer. Sections were then incubated with the primary antibody, goat anti-mouse UCP-1 antibody (1:100 dilution) (Santa Cruz Biotechnology, Inc., CA., USA), at 4 °C overnight. Sections were then washed in PBS, and immunostaining was identified using DAB peroxidase substrate solution as the chromogen. Tissue sections were counterstained with Mayer's hematoxylin solution, rinsed in running tap water, dehydrated and mounted with permanent mounting medium. Control sections were incubated with PBS in place of the primary antibody. Sections with primary antibody omitted in staining were used as controls. UCP-1 positive adipocyte area was counted under microscope (Olympus, CKX41) with the software Image-Pro Plus.

2.4. Preparation of aortic rings and contractility experiments

The procedure for the preparation of aortic rings has been described in our previous report (Gao and Lee, 2001; Gao et al., 2005a). Briefly, the thoracic aorta was collected in oxygenated physiological salt solution with the following composition (in mM): NaCl, 119; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; $CaCl_2$, 1.6; glucose, 11, at 4 °C. Paired aortic rings with or without PVAT (PVAT+ and PVAT−, 4 mm long for each) were prepared with either intact endothelium (E+) or with endothelium removed (E−) from the middle part of the thoracic aorta where PVAT is evenly distributed. To prepare E− rings, endothelium was removed by gently rubbing the internal surface with a fine wooden stick, and successful removal of endothelium was confirmed by the absence of a relaxation response to carbamycholine chloride (1 μM) in rings precontracted with phenylephrine (PHE, 1 μM). A computerized myograph system was used to record the isometric tension of the aortic rings. After equilibration for at least 90 min at 3 g of preload, which is the optimal preload defined in our previous experiments (Gao and Lee, 2001), the arterial rings were challenged with 60 mM KCl twice at an interval of 30 min. Contractile response was expressed as a percentage of KCl contraction, and relaxation response was calculated as a percentage of pre-contraction load. Viability of the vessels was tested with KCl at the end of experiment. The concentration of PHE required to induce 50% maximal response (EC_{50}) was estimated by fitting each concentration–response curve.

2.5. Bioassay experiments with thoracic aorta

To examine the effects of transferable relaxation factor from PVAT, bioassay experiments were carried out using PVAT+ aortic rings as donors, and PVAT− rings with or without endothelium as recipients. The transfer of solution incubated with PVAT− rings served as control. The donor and recipient vessels were pre-contracted with PHE (0.3 μM), and 3 ml of donor solution was transferred to the recipient chamber when the pre-contraction reaches its plateau (usually within 3–5 min), as described in previous studies (Gao et al., 2005a,b). To test the involvement of angiotensin-(1–7), recipient vessels were incubated with D-Ala^7-angiotensin 1–7 (A779, a Mas receptor antagonist) for 25–30 min before transfer of solution was carried out. An equal amount of A779 was added to the donor solution to avoid any dilution of this antagonist in the recipient chamber when donor solution was introduced.

2.6. Chemicals

The following chemicals were used: carbamycholine chloride and PHE were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), A779 was from Bachem Chemical Company (St. Louis, Missouri, USA).

2.7. Statistical analysis

All values were expressed as means ± S.E.M., where n represents the number of rats. Statistical analysis was performed by one-way ANOVA for comparison between rat groups (SHR versus WKY rats). In bio-assay experiments, a paired *t*-test was used to compare the relaxation responses between control and treatment with A779 from the same recipient vessel. The differences were considered significant when $P<0.05$.

3. Results

3.1. Blood pressure, body weight, and body fat contents

Systolic blood pressure was significantly higher, while body fat mass and its ratio to body weight were lower in SHR than in age-matched WKY rats (60–66 weeks of age). The lean mass (which includes bones and muscles) and its ratio to body weight were higher in SHR than in WKY rats. Body weight of SHR and WKY rats was similar (Table 1).

Table 1
Systolic blood pressure, body fat mass and non-fat mass in SHR and WKY rats at the age of 60–66 weeks.

Species	Systolic BP (mm Hg)	Body Weight (g)	Fat Mass (g)	Fat mass (% Total mass)	Lean Mass (g)	Lean mass (% Total mass)
SHR	190 ± 5.5 [a]	433 ± 6.2	56 ± 3.4[a]	13 ± 0.7 [a]	377 ± 5.7 [a]	87 ± 0.7 [a]
WKY	138 ± 3.3	448 ± 9.7	94 ± 6.7	21 ± 1.1	354 ± 5.8	79 ± 1.1

[a] $P<0.01$ compared to respective WKY rats (n = 5–8 rats for each group).