Life Sciences xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences

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

Obesity and heterozygous endothelial overexpression of prepro-endothelin-1 modulate responsiveness of mouse main and segmental renal arteries to vasoconstrictor agents

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ARTICLE INFO

Article history:
Received 1 November 2013
Accepted 27 December 2013
Available online xxxx

Chemical compounds studied in this article:
L-NAME hydrochloride
(PubChem CID: 135193)
ET-1 (PubChem CID: 16212950)
Serotonin hydrochloride
(PubChem CID: 160436)
15-Hydroxy-11 alpha,9
alpha-(epoxymethano)prosta-5,
13-dienoic acid
(U46619, PubChem CID: 5311493)

Keywords:
Endothelium
ET-1
Serotonin
TP receptor
U46619

ABSTRACT

Aims: Levels of the endothelium-derived peptide endothelin-1 (ET-1) are elevated in obese humans, and ET-1 mediated vascular tone is increased. Renal arterial smooth muscle is highly responsive to ET-1. Whether or not endothelium-derived ET-1 affects contractions of the renal artery under normal conditions or in obesity is unknown. The present study was designed to investigate whether or not overexpression of endogenous ET-1 in the endothelium affects the responsiveness of the main and segmental renal arteries differently in obesity.
Main methods: Mice with *tie-1* promoter-driven endothelium-restricted heterozygous overexpression of preproendothelin-1 were used (TET^{het}). Obesity was induced in TET^{het} mice and wild-type (WT) littermates by feeding a high fat diet for 30 weeks; lean controls were kept on standard chow. The renal arteries were studied in wire myographs testing contractions (in the presence of L-NAME) to ET-1, serotonin, and U46619.
Key findings: Contractions to ET-1 were comparable between groups in main renal arteries, but augmented in segmental preparations from obese mice. Serotonin-induced responses were enhanced in obese TET^{het} mice renal arteries compared to lean controls. Concentration–contraction curves to U46619 were shifted significantly to the left in main renal arteries of obese animals, and the maximal response was significantly increased between lean and obese TET^{het} mice.
Significance: These results indicate an augmented responsiveness of main renal arteries in obesity particularly to TP receptor activation. When combined with endothelial ET-1 overexpression this effect is even more pronounced, which may help to gain further insights into the mechanisms of hypertension in obesity.

Introduction

Kidneys play a key role in the homeostasis of salts and water and in the development of hypertension (Guyton et al., 1972). A reduction in renal perfusion leads to increased blood pressure (Goldblatt et al., 1934; Hall, 2003). The potent vasoconstrictor peptide endothelin-1 [ET-1 (Yanagisawa et al., 1988)] has been implicated in renal disease leading to high arterial blood pressure (Kohan et al., 2011). However, renal overexpression of either ET-1 or ET-2 does not cause hypertension per se (Hocher et al., 1996, 1997; Shindo et al., 2002). Nevertheless, such transgenic animals are prone to developing kidney fibrosis and salt-dependent hypertension (Hocher et al., 1997; Liefeldt et al., 1999; Shindo et al., 2002).

Obesity due to the imbalance between excessive calorie intake and reduced physical activity is one of the emerging global health issues and is associated with an activated endothelin system in humans with or without hypertension (Cardillo et al., 2004; Weil et al., 2011). The impact of obesity on responsiveness in the renal vascular bed is largely unknown. Therefore, the present experiments were designed to compare vasoconstrictor responses to ET-1, the platelet product serotonin and the thromboxane prostanoid (TP) receptor agonist U46619 in isolated main and segmental renal arteries of lean and obese mice. To determine whether or not increased levels of endothelial ET-1 would affect these responses, preparations from animals with heterozygous endothelium-restricted overexpression

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http://dx.doi.org/10.1016/j.lfs.2013.12.214

of the preproET-1 gene (Leung et al., 2004, 2011) were compared with those of wild-type (WT) littermates.

Materials and methods

Animals

Male mice with heterozygous endothelial overexpression of the murine preproET-1 cDNA (Leung et al., 2004) (TET^{het}) and wild-type (WT) littermates on a C57BL/6N background were housed in the laboratory animal unit at constant temperature under a 12 h light–dark cycle with free access to water and diet. Obesity was induced by a high fat diet (D12079B, Research Diets Inc., New Brunswick, NJ, USA) for 30 weeks (Traupe et al., 2002; Mundy et al., 2007), and lean control animals were kept on standard rodent chow (D5053, Lab Diet, Purina Mills, Richmond, IN, USA). The salt content was approximately 0.75% in the standard and 0.25% in the high fat diet. On the day of experiments mice were anesthetized with a mixture of fentanyl citrate (0.4 mg/kg), and fluanisone (12.5 mg/kg) plus midazolam (6.25 mg/kg) prior to exsanguination by cardiac puncture. Procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals issued by the U.S. Institute of Laboratory Animal Research (ILAR, eighth edition, 2011) and were approved by the institutional Committee on the Use of Live Animals for Teaching and Research of the University of Hong Kong.

Blood pressure and body fat

Systolic arterial blood pressure was determined non-invasively by the tail-cuff method (BP-2000 Blood Pressure Analysis System, Visitech Systems Inc., Raleigh, NC, USA) at the end of the study in conscious animals, following a training period of five days; such determination shows a high correlation with invasive measurements in unrestrained, unanesthetized mice (Krege et al., 1995). Body fat mass was determined in conscious mice by time-domain NMR spectroscopy (Minispec model LF90II, Bruker Instruments, Billerica, MA, USA).

Tissue harvesting and preparation

After sacrifice, the main renal arteries were dissected free of perivascular adipose and connective tissue. Rings of the left and right main and of segmental [first branch] renal arteries were suspended between 40 μM stainless steel wires in Halpern–Mulvany myographs (models 610 M and 620 M, Danish Myo Technology A/S, Aarhus, Denmark) filled with modified Krebs–Ringer bicarbonate solution [in mmol/L: NaCl, 129; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $NaHCO_3$, 14.9; glucose, 5.5; calcium disodium EDTA, 0.026; $CaCl_2$, 2.5 (control solution)] permitting to achieve a stable pH of 7.4 in the covered and aerated [95% O_2 plus 5% CO_2] chambers (Baretella et al., 2013). The preparations were equilibrated at 37° C for 30 min prior to the first application of force (1 mN). For each individual ring every following 1 mN increase in tension was followed by replacement of the control solution for a few minutes with an iso-osmotic high potassium solution (60 mmol/L high K^+ with equimolar substitution of sodium by potassium). The high K^+ solution was washed out after the induced response has exceeded or was similar in amplitude to the preceding high K^+ contraction resulting in an active tension–tension relationship (Vanhoutte and Leusen, 1969; Baretella et al., 2013). The response to the final depolarization with 60 mmol/L high K^+ was allowed to plateau, which occurred within two (segmental arteries) to ten (main renal arteries) minutes. This submaximal response was used as reference contraction.

Functional protocols

In order to exclude any effects of nitric oxide (NO), which modulate both the production and the action of ET-1 (de Nucci et al., 1988; Boulanger and Lüscher, 1990; Vanhoutte, 2000; Félétou et al., 2012) and the release of which could be initiated by ET-1 or serotonin acting on ET_B or 5-HT_{1D} receptors, respectively (Cohen et al., 1983; de Nucci et al., 1988; Schoeffter and Hoyer, 1990; Schini et al., 1991; Hirata et al., 1993), all experiments were performed in the presence of a NO synthase inhibitor. The rings were equilibrated for at least ten minutes prior to incubation with the non-selective nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 3×10^{-4} mol/L) for 30 min to block basal and receptor-stimulated generation of NO (Widmer et al., 2006; Baretella et al., 2013). The left renal artery was exposed to a full concentration-response curve of serotonin (10^{-10} to 3×10^{-5} mol/L); rings of the right renal artery were contracted with ET-1 (10^{-11} to 10^{-7} mol/L) and U46619 (10^{-11} to 3×10^{-6} mol/L). If consecutive contractions were obtained with different agonists, the preparations were relaxed maximally with sodium nitroprusside (10^{-5} mol/L), followed by several washouts prior to re-incubation with L-NAME and addition of the next drug.

Drugs

N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), serotonin and sodium nitroprusside were purchased from Sigma-Aldrich (St. Louis, MO, USA); 9,11-Dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619) and endothelin-1 (ET-1) were from Enzo Life Sciences (Farmingdale, NY, USA). All drugs were dissolved in distilled water except U46619, which was dissolved in ethanol (10^{-2} mol/L; final concentrations in the myograph chamber not exceeding 0.1%). Stock solutions for ET-1 (10^{-5} mol/L) were dissolved in 5% acetic acid (100 μl aliquots). Concentrations are given as final molar concentration in the myograph chamber solution.

Statistical analysis

All contractions are expressed as percentage of the reference contraction to 60 mmol/L high K^+. Maximal contractions (E_{max}) and concentrations causing half maximal responses (EC_{50}; shown as pD_2 values) were calculated and compared using nonlinear regression analysis in Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). Data were tested for distribution normality using the D'Agostino–Pearson test. Student's *t*-test for unpaired observations or the Mann–Whitney *U* test were applied to analyze statistical differences between groups of normally distributed or non-parametric samples, respectively. Two-way analysis of variance (ANOVA) for multiple comparisons followed by Bonferroni post hoc test was used where appropriate. Results are shown as means ± standard error of the mean (SEM), and *n* equals the number of preparations from different animals. *P* values smaller than 0.05 were considered to indicate statistically significant differences.

Results

Obesity development and blood pressure

All animals on high fat diet had developed obesity after 30 weeks treatment compared to lean mice kept on standard chow (fat mass: 5.4 ± 0.7 g in lean WT, 5.5 ± 0.7 g in lean TET^{het} mice, 15.4 ± 0.5 g in obese WT and 16.8 ± 0.7 g in obese TET^{het}). Systolic arterial blood pressure did not differ significantly irrespective of genotype between lean and obese animals and was within normal limits (lean WT: 107.1 ± 2.3 mm Hg, lean TET^{het}: 103.7 ± 2.8 mm Hg, obese WT: 116.1 ± 4.4 mm Hg, obese TET^{het}: 105.7 ± 3.5 mm Hg).

Functional studies

Submaximal contractions to potassium chloride (60 mmol/L high K^+) averaged 6.9 ± 0.6 mN for the left and 8.5 ± 0.5 mN in the right main renal artery; these reference contractions were comparable between groups (data not shown). The reference contractions of

Please cite this article as: Baretella O, et al, Obesity and heterozygous endothelial overexpression of prepro-endothelin-1 modulate responsiveness of mouse main and segmental renal..., Life Sci (2014), http://dx.doi.org/10.1016/j.lfs.2013.12.214