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Cyclooxygenase-2-derived prostanoids reduce inward arterial remodeling induced by blood flow reduction in old obese Zucker rat mesenteric arteries

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ABSTRACT

Obesity is associated with altered arterial structure and function leading to arterial narrowing in most vascular beds, especially when associated with aging. Nevertheless, mesenteric blood flow remains elevated in obese rats, although the effect of aging remains unknown. We investigated mesenteric artery narrowing following blood flow reduction in vivo in 3- and 12-month-old obese Zucker rats.

After 21 days, inward remodeling occurred in low flow (LF) arteries in young and old lean rats and in young obese rats (30% diameter reduction). Diameter did not significantly decrease in old obese rats. Phenylephrine-mediated contraction was reduced by approximately 20% in LF arteries in all groups but in old obese rat arteries in which the decrease reached 80%. LF arteries expressed cyclooxygenase-2 and blood 6-keto-PGF1alpha (prostacyclin metabolite) was elevated in old obese rats. In old obese rats, acute cyclooxygenase-2 blockade restored phenylephrine-mediated contraction in LF arteries and chronic cyclooxygenase-2 blockade restored inward remodeling and contractility to control level.

Thus, in old obese rats, cyclooxygenase-2-derived prostacyclin prevented the diameter reduction induced by a chronic decrease in blood flow. This adaptation is in favor of a preserved perfusion of the mesentery by contrast with other vascular territories, possibly amplifying the vascular disorders occurring in obesity.

1. Introduction

The metabolic syndrome is a major health problem affecting a large proportion of the population worldwide. Its incidence increases in parallel to the enhanced prevalence of obesity and diabetes (Herman and Zimmet, 2012; Rao et al., 2012; Wild et al., 2004). The metabolic syndrome is associated with an increasing risk of overall mortality as well as cardiovascular morbidity and mortality (Hu et al., 2004). It is defined as an association of three or more of the following risk factors: impaired glucose tolerance or insulin resistance, increased blood pressure, increased plasma triglycerides and/or low HDL cholesterol, obesity, microalbuminuria (Alberti and Zimmet, 1998; Onat, 2011; Prasad et al., 2012). The metabolic syndrome is associated with endothelial dysfunction (Goodwill and Frisbee, 2012; Thomas et al., 2004) and vascular remodeling (Rocic, 2012) leading to arterial wall hypertrophy (Iannuzzi et al., 2005). This vascular dysfunction is most likely involved in the higher incidence of end-organ damage in patients with metabolic syndrome.

Resistance arteries play a key role in the control of local blood flow and their capacity to adapt in response to chronic changes in blood flow is essential after ischemia as well as in response to exercise (Henrion, 2005; van den Akker et al., 2009; Vessieres et al., 2012). In obese Zucker rats (OZR), vasoconstrictor tone increases and NO-dependent dilation decreases in the renal circulation (Stepp et al., 2007). By contrast, blood flow in the mesenteric circulation is higher in OZR than in lean animals (Enevoldsen et al., 2000; Romanko and Stepp, 2005). Despite reduced NO-dependent dilation in OZR mesenteric arteries (Bouvet et al., 2007; Romanko and Stepp, 2005), vasoconstrictor tone is also reduced, thus preserving mesenteric blood flow in hyperphagic rats (Romanko and Stepp, 2005). We have recently shown that the diameter enlargement induced by a chronic increase in blood flow, which is impaired in one-year old lean rats, is maintained in old OZR (Belin de Chantemele et al., 2010). This finding suggests that OZR mesenteric arteries remain able to further enlarge and thus to support higher blood flow. Nevertheless, when metabolic syndrome develops with age, rats become heavier with more fat, especially in the mesentery and they become

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moderately, although significantly, hypertensive and diabetic (Belin de Chantemele et al., 2010). In these conditions blood flow in mesenteric arteries, unlike in other vascular beds, should decrease and thus arteries should undergo inward remodeling as observed in both hypertension (Prewitt et al., 2002) and diabetes (Schofield et al., 2002). Nevertheless, inward remodeling depends on the basal constrictor tone of the arteries and the local inflammation affecting the mesentery might maintain an excessive vasodilatation. Thus, we investigated the effect of aging and metabolic syndrome on the capacity of mesenteric arteries to develop inward eutrophic remodeling in response to a chronic decrease in blood flow. Young and old obese Zucker rats (OZR) were used as a model of metabolic syndrome.

We used a model previously described (Freidja et al., 2012; Loufrani et al., 2002a; Pourageaud and De Mey, 1997; Tarhouni et al., 2013) based on ligation of mesenteric arteries. This model allows the comparison of resistance arteries submitted chronically to low or normal blood flow in the same vascular bed and in the same physiological conditions in vivo. Blood flow decreases by 80% in the ligated arteries leading to inward eutrophic remodeling (Belin de Chantemele et al., 2009; Freidja et al., 2011; Loufrani et al., 2002b). We used 12-month old obese Zucker rats, which are relatively old as they have a limited life expectancy (Johnson et al., 1997).

2. Materials and methods

2.1. Animals

The procedure followed in the care and euthanasia of the study animals was in accordance with the European Community Standards on the Care and Use of Laboratory Animals (Ministère de l'Agriculture, France, authorization No. 6422). The protocol is approved by the Committee on the Ethics of Animal Experiments of the "Pays de la Loire" Region ("Comité d'éthique en Expérimentation Animale", CEEA, permit # CEEA PdL 2008.10).

Three and 12-month old male obese Zucker rats (OZR) and lean Zucker rats (Charles River, L'Arbresles, France) were anesthetized (isoflurane, 2.5%) and pre-treated with buprenorphine (Temgesic®; 0.1 mg/kg, s.c.). A loop of intestine was then exposed and local mesenteric artery blood flow was surgically reduced, as previously described (Belin de Chantemele et al., 2009). The ligated artery was designed as low flow (LF) arteries. Other arteries located at the distance of the ligated arteries were used as control arteries (normal flow, NF).

Twenty-one days after surgery, animals were anesthetized (Isoflurane, 2.5%). The left femoral artery was cannulated for blood pressure measurement as previously described (Driss et al., 2000). Blood samples were then collected and the rats were sacrificed by CO_2 inhalation. The gut was excised and the mesenteric arteries were gently dissected. From each rat, LF and NF arteries were isolated and divided in several segments used respectively for pressure–diameter relationship measurement, pharmacology as well as for immunohistological and biochemical analyses. Twelve rats were used per group and each experiment described below was performed on a different segment of artery obtained from each animal.

In another series of experiments, 12-month-old lean rats and OZR were treated with the COX-2 inhibitor celecoxib (25 mg/kg, forced feeding, twice daily) (Belin de Chantemele et al., 2010).

2.2. Blood and plasmatic parameters

Before sacrifice, glycemia was quantified on a sample of arterial blood with a glucometer as previously described (Belin de Chantemele et al., 2010). Plasma dinor-6-keto PGF1alpha was then determined as previously described (Retailleau et al., 2010) using a commercially available kit (Cayman).

2.3. Pressure–diameter relationship and histomorphometry in isolated arteries

Arterial segments were cannulated at both ends and mounted in a video monitored perfusion system as previously described (Bolla et al., 2002; Retailleau et al., 2013). Briefly, cannulated arterial segments were bathed in a 5 ml organ bath containing a Ca^{2+}-free physiological salt solution containing ethylenbis-(oxyethylenenitrolo) tetra-acetic acid (EGTA, 2 mmol/L) and sodium nitroprusside (10 μmol/L). Pressure steps (10 to 150 mm Hg) were then performed in order to determine passive arterial diameter. Pressure and diameter measurements were collected using a Biopac data acquisition system (Biopac MP100 and Acqknowledge® software; La Jolla, CA, USA). The arterial segment was then fixed with formaldehyde under a pressure of 75 mm Hg in order to measure media cross-section area and wall thickness as previously described (Loufrani et al., 2002b).

2.4. Contractility of isolated mesenteric arteries

Other segments of LF and NF mesenteric arteries (2 mm long) were mounted on a wire-myograph (DMT, Aarhus, DK) as previously described (Henrion et al., 1992). Briefly, 2 tungsten wires (40 μm in diameter) were inserted into the lumen of the arteries and fixed to a force transducer and a micrometer, respectively. Arteries were bathed in a physiological salt solution. Wall tension was applied as described previously (Dowell et al., 1996).

Cumulative concentration–response curve (CRC) to phenylephrine (0.001 to 10 μmol/L) was performed before and after incubation with the cyclooxygenase inhibitor indomethacin (10 μmol/L, 20 min) or with the selective COX-2 inhibitor NS398 (10 μmol/L, 20 min) (Henrion et al., 1996).

2.5. Western blot analysis

Other arterial segments were pooled and then homogenized. Proteins (25 μg total protein from each sample) were separated by SDS-PAGE using a 4% stacking gel followed by a 10% running gel. Proteins were detected with specific antibodies (Santa Cruz Biotechnology, COX-2 1:500, sc-1746 and beta-actin 1:1000 in TBST). Protein expression was visualized using the Luminol Reagent (Santa Cruz Biotechnology) (Baron-Menguy et al., 2010).

2.6. Immunohistological analysis of COX-2

Arterial segments were mounted in embedding medium (Tissu-Tek, Miles, Inc), frozen in isopentane pre-cooled in liquid nitrogen, and stored at −80 °C. COX-2 was detected on transverse cross sections (7 μm thick) using primary goat anti-COX-2 polyclonal antibodies (1/100, Santa Cruz Biotechnology, sc-1747) followed by the fluorescent secondary antibody (1/200, Fluoroprobes) as previously shown (Retailleau et al., 2010). In negative control experiments the primary antibody was omitted. A positive control experiment was performed using a similar artery obtained from a rat treated with lipopolysaccharide.

2.7. Statistical analysis

Results are expressed as means ± SEM. Significance of the difference between arteries was determined by ANOVA (1-factor ANOVA or ANOVA for consecutive measurements, when appropriate). Means were compared by paired *t*-test or by the Bonferroni test for multigroup comparisons. Values of $P < 0.05$ were considered to be significant.