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Functional heterogeneity of NADPH oxidase-mediated contractions to endothelin with vascular aging

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ABSTRACT

Aims: Aging, a physiological process and main risk factor for cardiovascular and renal diseases, is associated with endothelial cell dysfunction partly resulting from NADPH oxidase-dependent oxidative stress. Because increased formation of endothelium-derived endothelin-1 (ET-1) may contribute to vascular aging, we studied the role of NADPH oxidase function in age-dependent contractions to ET-1.
Main methods: Renal arteries and abdominal aortas from young and old C57BL6 mice (4 and 24 months of age) were prepared for isometric force measurements. Contractions to ET-1 (0.1–100 nmol/L) were determined in the presence and absence of the NADPH oxidase-selective inhibitor gp91ds-tat (3 μmol/L). To exclude age-dependent differential effects of NO bioactivity between vascular beds, all experiments were conducted in the presence of the NO synthase inhibitor L-NAME (300 μmol/L).
Key findings: In young animals, ET-1-induced contractions were 6-fold stronger in the renal artery than in the aorta ($p < 0.001$); inhibition of NADPH oxidase by gp91ds-tat reduced the responses to ET-1 by 50% and 72% in the renal artery and aorta, respectively ($p < 0.05$). Aging had no effect on NADPH oxidase-dependent and -independent contractions to ET-1 in the renal artery. In contrast, contractions to ET-1 were markedly reduced in the aged aorta (5-fold, $p < 0.01$ vs. young) and no longer sensitive to gp91ds-tat.
Significance: The results suggest an age-dependent heterogeneity of NADPH oxidase-mediated vascular contractions to ET-1, demonstrating an inherent resistance to functional changes in the renal artery but not in the aorta with aging. Thus, local activity of NADPH oxidase differentially modulates responses to ET-1 with aging in distinct vascular beds.

Introduction

Endothelin-1 (ET-1) is the predominant isoform of three distinct isopeptides constitutively secreted by endothelial and other vascular cells, and the most potent endogenous vasoconstrictor known (Yanagisawa et al., 1988; Kohan et al., 2011). The renal artery is particularly sensitive to ET-1 (Clozel & Clozel, 1989; Pernow et al., 1989; Widmer et al., 2006), and an increase in renal artery tone may lead to reduced kidney perfusion and subsequent activation of the renin–angiotensin system, which contributes to the ET-1-dependent regulation of basal vasomotor tone and blood pressure (Kohan et al., 2011; Haynes & Webb, 1994; Barton & Shaw, 1997). However, ET-1 also induces vascular oxidative stress, inflammation and remodeling (Amiri et al., 2004, 2008). Indeed, ET-1 contributes to vascular stiffening and calcification with aging, which are all major independent cardiovascular risk factors and associated with cardiovascular complications such as myocardial infarction, stroke, and renal injury (Zieman et al., 2005).

ET-1 activates two G protein-coupled receptors, ET_A and ET_B (Kohan et al., 2011). In the vascular wall, smooth muscle cells predominantly express ET_A receptors to mediate vasoconstriction, although contractions in response to ET_B receptor activation have also been reported for some vascular beds (Kohan et al., 2011). However, ET_B receptors are predominantly found on endothelial cells, where their activation results in the release of the vasodilators nitric oxide (NO) and prostacyclin; moreover, ET_B receptors are important for ET-1 clearance (Kohan et al., 2011). ET-1 acts in concert with other endothelium-derived contracting factors to balance the activity of endothelium-derived relaxing factors (Feletou & Vanhoutte, 2006). However, vascular aging impairs endothelial cell function favoring the production of contracting factors, including ET-1 (Barton, 2010; Seals et al., 2011). We have previously shown that aging increases circulating ET-1 levels, functional

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endothelin converting enzyme activity in the aorta, as well as ET-1 expression in conduit and renal arteries of otherwise healthy, normotensive animals (Barton et al., 1997; Goettsch et al., 2001). Accordingly, aging augments endothelial ET-1 expression (Donato et al., 2009) and ET-1-dependent vascular tone in human arteries (Van Guilder et al., 2007; Thijssen et al., 2007; Westby et al., 2011). These findings point towards an increase in ET-1 bioactivity with vascular aging, as also evidenced from the increased exocytotic ET-1 release in aged endothelial cells (Goel et al., 2010).

Many of the detrimental effects of vascular aging have been attributed to the increased generation of oxygen-derived free radicals, particularly superoxide (Barton, 2010; Seals et al., 2011; Oudot et al., 2006; Donato et al., 2007). Although reactive oxygen species can stimulate ET-1 production, ET-1 on the other hand may also induce superoxide generation by activating NADPH oxidase (Pollock & Pollock, 2005). In young rats, ET-1 enhances NADPH oxidase activity in carotid arteries (Li et al., 2003), and induces contractions of renal arteries and aorta that are partly mediated by NADPH oxidase-derived superoxide (Loomis et al., 2005; Just et al., 2008). Moreover, activation of vascular NADPH oxidase is likely involved in impaired endothelium-dependent vasodilation and vascular remodeling due to ET-1 overproduction in transgenic mice (Amiri et al., 2004). Likewise, NADPH oxidase has been identified as an important source of ET-1 stimulated superoxide production in mammary arteries and saphenous veins of patients with coronary artery disease (Cerrato et al., 2012). These findings suggest that generation of NADPH oxidase-derived superoxide may contribute to the ET-1-dependent regulation of vascular homeostasis in physiology and disease.

It is however not known whether vascular aging affects contractile responses to ET-1 mediated by NADPH oxidase. Given the physiological importance and high sensitivity of the renal vasculature to ET-1 (Kohan et al., 2011; Clozel & Clozel, 1989; Pernow et al., 1989; Widmer et al., 2006), the present study was therefore designed to determine whether age affects ET-1-induced contractions, particularly through NADPH oxidase, in the renal artery. Parallel experiments were conducted in the aorta, which has previously been shown to be sensitive to ET-1-related vascular aging (Barton et al., 1997; Goettsch et al., 2001).

Materials and methods

Materials

ET-1 was from American Peptide (Sunnyvale, CA, USA), the NADPH oxidase-selective inhibitor gp91ds-tat (Rey et al., 2001) from Anaspec (Fremont, CA, USA), and the NO synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME) from Cayman Chemical (Ann Arbor, MI, USA). All other drugs were from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared according to the manufacturer's instructions, and diluted in physiological saline solution (PSS, composition in mmol/L: 129.8 NaCl, 5.4 KCl, 0.83 $MgSO_4$, 0.43 NaH_2PO_4, 19 $NaHCO_3$, 1.8 $CaCl_2$, and 5.5 glucose; pH 7.4) to the required concentrations before use.

Animals

Young and old male C57BL6 mice (4 and 24 months of age, mean body weight 29 ± 1 g and 31 ± 1 g, respectively, Harlan Laboratories, Indianapolis, IN) were bred and housed at the animal research facility of the University of New Mexico Health Sciences Center. Animals had free access to standard rodent chow and water, with a 12 hour light–dark cycle. All procedures were approved by the University of New Mexico Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Isolated vessel preparation

After mice were euthanized by intraperitoneal injection of sodium pentobarbital (2.2 mg/g body weight), renal arteries and the abdominal aorta were immediately excised and transferred into cold (4 °C) PSS. Vessels were carefully dissected free from adherent connective tissue and fat, cut into rings 2 mm in length, and transferred to organ chambers of a Mulvany–Halpern myograph (620M Multi-Channel Myograph System, Danish Myo Technology, Aarhus, Denmark) containing PSS. Renal artery rings were mounted using two 25 μm tungsten wires threaded through the vessel lumen and secured to mounting jaws, whereas abdominal aorta rings were transferred onto 200 μm stainless steel pins. The jaws or pins were connected either to a micropositioner or to a force transducer for the recording of isometric tension.

Vascular pharmacology studies

After equilibrating for 30 min in PSS (37 °C, pH 7.4, bubbled with 21% O_2, 5% CO_2 and balanced N_2), vascular rings were stretched stepwise until the optimal passive tension for generating force during isometric contraction was reached. Vessels were equilibrated for an additional period of 30 min (renal artery) or 45 min (abdominal aorta), and repeatedly exposed to K^+ (PSS with equimolar substitution of 60 mmol/L potassium for sodium) to confirm vascular smooth muscle integrity and to determine maximal contractile responses. The role of NADPH oxidase was studied by randomly treating the left or right renal artery as well as one of two neighboring rings of the abdominal aorta with the Nox-selective inhibitor gp91ds-tat (3 μmol/L for 30 min) (Rey et al., 2001; Park et al., 2004; Miller et al., 2005). Gp91ds-tat consists of a 9-amino acid peptide of the Nox1/Nox2 catalytic subunits of NADPH oxidase (at the interface with $p47^{phox}$, which is essential for activity) linked to the 11-amino acid HIV-tat peptide, which facilitates cellular entry (Rey et al., 2001; Brandes et al., 2010). After the incubation period, vessels were exposed to cumulative concentrations of ET-1 (0.1–100 nmol/L) or to the predominantly α_1-adrenergic agonist phenylephrine (1 μmol/L). All experiments were performed following inhibition of NO synthase by L-NAME (300 μmol/L for 30 min) to unmask contractions in the aorta (Widmer et al., 2006), and to exclude ET_B receptor-stimulated NO release (Kohan et al., 2011) as well as potential differences in NO bioavailability between vascular beds and age groups (Barton, 2010; Seals et al., 2011).

Data calculation and statistical analyses

Data are expressed as mean ± SEM; *n* equals the number of animals used. Contractions to ET-1 are given relative to K^+ (60 mmol/L)-induced responses. Fitting of dose–response curves to calculate area under the curve (AUC), EC_{50} values (as negative logarithm, pD_2) and maximal responses was performed as described by DeLean et al. (1978). Data was analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test (Prism version 5.0 for Macintosh, GraphPad Software, San Diego, CA, USA). A $p < 0.05$ value was considered significant.

Results

The renal artery is resistant to ET-1-related functional aging

To study the functional effects of aging on ET-1-dependent vascular tone, we first determined contractile responses in young and old mice (4 and 24 months of age). ET-1 induced potent contractions in the renal artery of young animals that were 6-fold stronger compared to the abdominal aorta (102 ± 4% vs. 18 ± 4%, $n = 4–8$, $p < 0.001$, Fig. 1A). In the aorta, aging reduced contractions to ET-1 by 78% (from 18 ± 4% to 4 ± 1%, $n = 5–8$, $p < 0.01$, Fig. 1A), whereas there was no change in the renal artery (102 ± 4% vs. 92 ± 8%, $n = 4–5$, $p =$ n.s.

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