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Paraoxonase1 deficiency in mice is associated with hypotension and increased levels of 5,6-epoxyeicosatrienoic acid

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ABSTRACT

Aim: Serum paraoxonase 1 (PON1) is an HDL-associated lipolactonase and its association with hypertension is controversial. We studied the possible role of PON1 in blood pressure (BP) regulation, by using PON1 knockout (PON1KO) mice.
Methods and results: Both, systolic and diastolic BPs were lower in PON1KO compared to WT mice. Hypotension detected in PON1KO is probably neither related to nitric oxide/guanylate cyclase-mediated vasodilation nor to angiotensin II or aldosterone-mediated vasoconstriction. Surprisingly, when challenged by high-salt diet, BP was further reduced in PON1KO mice. The later, pointed to a possible involvement of transient receptor potential vanilloid 4 (TRPV4), and indeed, administration of ruthenium red, a TRPV4 blocker, resulted in a sharp rise in BP. The protein levels of TRPV4 in kidneys of PON1KO were not higher than in WT. However, the renal level of 5,6-epoxyeicosatrienoic acid (5,6-EET), a TRPV4 specific agonist, was significantly higher in PON1KO compared with WT mice. 5,6-EET levels were further elevated under high-salt diet or administration of arachidonic acid. Injection of inhibitor of CYP450 epoxygenase resulted in increased BP in PON1KO mice. Injection of recombinant human PON1 resulted in elevation of BP and a concomitant reduction in renal content of 5,6-EET. PON1, in vitro, metabolized 5,6-EET, but not other EETs, to its corresponding diol. Vasodilation, blocked by excess of dietary K^+ but not reversed by depletion of cellular Ca^{2+} stores, point to endothelial-derived hyperpolarization-like response.
Conclusion: The present study shows causal, direct relationship between PON1 and blood pressure which is mediated, at least in part, by the regulation of 5,6-EET.

1. Introduction

Hypertension is a major risk factor for increased prevalence of cardiovascular diseases (CVD). Blood pressure regulation is mediated by multiple genetic and environmental factors such as the renin-angiotensin aldosterone system (RAAS), neurohormonal systems and dietary factors. Among the genetic factors claimed to play a role in the prevalence of CVD is the HDL-associated enzyme Paraoxonase 1 (PON1) [1]. PON1 is capable of hydrolyzing a diverse array of substrates, including a variety of esters, lactones and oxidized lipids. Yet, its natural physiological/pathological substrate is still unknown but it is likely to be a lipid lactone [2–4].

The protective role of PON1 against atherosclerosis development was demonstrated in studies, which used mice lacking PON1 (PON1KO) [5,6], overexpressing PON1 [7] or PON1-transfected macrophages [8]. The PON1 antiatherogenic properties include protection of LDL, HDL and macrophages against oxidative stress, attenuation of oxidized-LDL uptake by macrophages and inhibition of macrophage cholesterol biosynthesis (reviewed in [9]).

Although there have been suggestions that serum PON1 activity is inversely related to the risk for cardiovascular diseases [10,11], an increasing number of studies associate the PON1 192RR genotype which has higher activity, with increased risk for hypertension [12] and CVD [13,14].

The present study aimed to assess the involvement of PON1 in the regulation of BP and to gain an insight into potential underlying mechanisms by using PON1 knockout mice.

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2. Materials and methods

2.1. Animals

PON1 knockout mice (PON1KO) on the background of C57BL6 mice were obtained as previously described [15]. Male mice aged 10 weeks were used for experiments.

High salt diet (3% NaCl), was supplemented in the drinking water for two weeks. AngII (5 mg/ml) or aldosterone (1 mg/ml) administered at a rate of 0.12 μl/min via osmotic minipumps implanted in the peritoneum (1002 Alzet Osmotic Pumps, Cupernito, USA).

Two hundred units of arylesterase activity of recombinant human PON1 protein [16] (The Israel Structural Proteomic Center, Rehovot, Israel) were injected i.p. and i.m. (100 U each) arachidonic acid (0.1 mg/mouse) was injected i.p.

Animal study protocol was approved by the Committee for the Supervision of Animal Experiments of the Technion – Israel Institute of Technology (# IL-046-04-2008) and was conducted in accordance with Israel laws for animal care and conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

2.2. Blood pressure (BP) measurement

BP was measured in conscious mice with a tail-cuff (5 measurements per mouse) linked to an IITC-229 NIBP System and computer software IITC-31 (Life Science Instruments, Woodland Hills, CA, USA). Only BP measurements before and after i.v. injection of ruthenium red (RuR, 1 mg/kg) were taken under light anesthesia with isoflurane, where BP in anesthetized animals was not significantly different compared to conscious mice (data not shown).

2.3. Detection of TRPV4 protein expression by Western blot analysis

Frozen kidneys were homogenized, 40 μg protein samples were separated on a 10% sodium dodecyl sulfate–polyacrylamide gel, and transferred to a polyvinylidene fluoride membrane. Blocking of the membrane was performed with 2% BSA for 2 h at room temperature followed by exposure to primary antibody targeted against TRPV4 (1:500, Santa Cruz Biotechnology) and a secondary antibody conjugated with horseradish peroxidase (1:10,000, Sigma). The membranes were developed using an ECL kit (Amersham Pharmacia Biotech) and subjected to densitometry scanning and analysis with the use of the ImageQuant (LAS4010, GE Life Sciences, Wisconsin, USA). β-Actin was used to normalize protein loading on membranes.

2.4. Serum and urine analysis

Nitrites were measured using Griess reagent (Sigma). Guanosine 3′,5′-cyclic monophosphate (cGMP) was measured using immunoassay kit (R&D Systems, MN, USA). Plasma renin activity (PRA), serum Angiotensin II (AngII) were measured using RIA kit (DiaSorin, Saluggia, Italy) Aldosterone levels were determined using a commercial RIA kit (Coat-a-count aldosterone; DPC, Los Angeles, CA, USA).

2.5. Extraction of 5,6-epoxyeicosatrienoic acid (5,6-EET) from mice kidneys

Extraction of epoxyeicosatrienoic acids is based on method described by Falck et al. [17]. Frozen kidneys were homogenized in the presence of antioxidant. After addition of internal standard, samples were extracted in chloroform:methanol. The chloroform phase was collected, evaporated, and redissolved in methanol and acetonitrile for LC/MS analysis (please see online supplement).

2.6. Analysis of 5,6-epoxyeicosatrienoic acid (5,6-EET) by LC/MS

The LC/MS was equipped with an HPLC (Waters 2790) that had a Waters photodiode array detector (model 996) connected to MS (Micromass Quattro Ultima MS, UK). The eluents were gradient of increasing acetonitrile concentrations with 0.1% acetic acid. MS/MS analysis of the 5,6-EET was performed in daughter mode, using electro spray ionization at anion mode. A calibration curve of the 5,6-EET or other EETs (Cayman Chemicals, Michigan, USA) and of IS were run in each analysis. The recovery of the internal standard was above 90% (please see online supplement).

2.7. Statistical analysis

Results are expressed as mean ± SEM, $n = 5–7$ for each treatment group. Two-tailed Student's t-test was used to determine statistical significance when comparing two arrays of data. A $p < 0.05$ value is considered statistically significant.

3. Results

PON1KO mice exhibit a significantly lower BP compared with control mice (Fig. 1). Systolic and diastolic BPs were 15.2 and 7.3 mmHg lower in PON1KO vs. WT. Heart rate was not significantly different between the two groups, 497 ± 7 and 477 ± 14 beats/min for WT and PON1KO, respectively, indicating that adrenergic system is probably not involved.

Potentially, enhanced endothelial function as reflected by activation of the NO/cGMP relaxing pathway [18] could mediate vasodilatation-induced hypotension observed in PON1KO mice. To test this possibility, serum concentrations of nitrites and cGMP were measured. Serum nitrites levels were not significantly different between PON1KO and WT (Fig. 2A). Levels of serum cGMP were significantly ($p = 0.03$) lower by 33% in PON1KO compared to control mice (Fig. 2B). These results, do not support a role for the NO/cGMP relaxing mechanism in the decreased BP observed in PON1KO and might indicate that mediators other than NO are also involved in regulation of low BP in PON1KO mice.

Reduced vasoconstriction, rather than enhanced vasodilation could result in hypotension. Therefore, we next examined if the hypotension in PON1KO mice involves reduced RAAS. While PRA, and AngII were not significantly different between PON1KO and WT, serum aldosterone was significantly lower in PON1KO by 36% (Fig. 3A–C, respectively). The lower levels of aldosterone are probably attributed to the lower availability of cholesterol ester required for steroidal synthesis as we have recently reported [19]. Expression of the corresponding receptors in the kidney, namely the AngII receptor type I and the mineralocorticoid receptor, were not significantly different between PON1KO and WT mice (data not shown). Two weeks administration of AngII or aldosterone failed to increase BP in PON1KO mice (Fig. 3D). These results suggest that the hypotension in these mice is attributed to a mechanism other than reduced activity/expression of the major RAAS components.

Beyond vascular resistance, arterial pressure depends also on blood volume status. BP is expected to increase under salt loading due to sodium retention and extracellular fluid volume expansion. Surprisingly, challenging PON1KO mice with dietary salt intake resulted in further decrease in BP (Fig. 4A) with no change in heart rate (data not shown). Such paradoxical augmentation of hypotensive response to high salt challenge was recently reported to be associated with activation of the transient receptor potential vanilloid 4 (TRPV4) [20,21]. To determine whether this hypotensive response of PON1KO mice to high salt involves similar mechanism,