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Upregulation of ANP and NPR-C mRNA in the kidney and heart of eNOS knockout mice

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ABSTRACT

Objectives: The aim of the present studywas to examine the question of whether the atrial natriuretic peptide (ANP) system is altered by endothelial nitric-oxide synthase (eNOS).
Methods: Male eNOS-deficient mice (eNOS−/−) and wild type control mice (eNOS+/+, C57B1/6J) were used. Blood pressure was measured in anesthetized mice by tail cuff plethysmography and renal function was measured. Expression of ANP, natriuretic peptide receptor (NPR)-A, NPR-C, and tonicity-responsive enhancer binding protein (TonEBP) mRNA was determined by real-time PCR. Localization of ^{125}I-ANP binding sites was measured using in vitro autoradiography.
Results: In eNOS−/− mice, systolic blood pressure increased and left ventricular hypertrophy was observed. Urine volume and osmolarity did not change. Expression of ANP markedly increased in the heart and kidney of eNOS−/− mice. Expression of NPR-A and NPR-C increased in the heart and tended to increase in the kidney of eNOS−/− mice. In the renal medulla in particular, increased expression of NPR-C was more prominent. Expression of TonEBP mRNA was markedly decreased in the renal medulla, but not in the renal cortex. Maximum binding capacity (B_{max}) of ANP and C-ANP increased in the renal medulla in eNOS−/− mice.
Conclusion: These results suggest that the eNOS-NO system may be partly involved in regulation of ANP, NPR-A, -C, and TonEBP mRNA expression in the kidney.

1. Introduction

Atrial natriuretic peptide (ANP) and brain natriuretic peptide are secreted from cardiac myocytes, not only by some mechanical stimuli (such as the wall stretch) [15,29], but also by the action of some neuro-hormone and immune systems [10,12–14]. These natriuretic peptides exert diuretic, vasorelaxant, and antiproliferative activities by binding to natriuretic peptide receptor-A (NPR-A), which contains particulate guanylyl cyclase (GC) catalytic activity [17,28]. In contrast, NPR-C is a non-GC receptor, and is coupled to adenylyl cyclase (AC) inhibition or phospholipase C activation through inhibitory guanine nucleotide regulatory protein [2,18]. These receptors are distributed in a variety of tissues, including kidney, heart, and vascular smooth muscle. Although the biological functions of ANP largely depend on expression of NPRs mRNA and their proteins, their regulation has not been clear until now.

On the other hand, the renal medulla has a high capacity for production of nitric oxide (NO) under both physiological and pathological conditions [30]. All three NO synthase (NOS) isoforms (endothelial, neuronal, and inducible NOS) have been identified in the kidney [23,30] and endothelial NOS (eNOS) activity in the renal medulla is 25-fold higher than that in the cortex [23,30]. Renal medullary tonicity is highly variable by virtue of changes in body fluid balance and regulates many osmosensitive genes [11]. Expression of eNOS correlates inversely with extracellular tonicity [7,18,24]. It has been shown that hypertonicity induced by NaCl or sucrose, but not urea, increased NPR-A activity, gene expression, and promoter activity in inner medullary collecting duct (IMCD) cells, which compose the terminal segment of the nephron responsible for final regulation of urinary sodium concentration [8]. Increased extracellular tonicity causes reduction of eNOS protein level, mRNA, and gene promoter [7,8]. From several series of experiments, Chen et al. concluded that reduction in eNOS expression with concomitant decline in cGMP levels accounted for osmotic stimulation of the NPR-A gene [9]. We have recently shown

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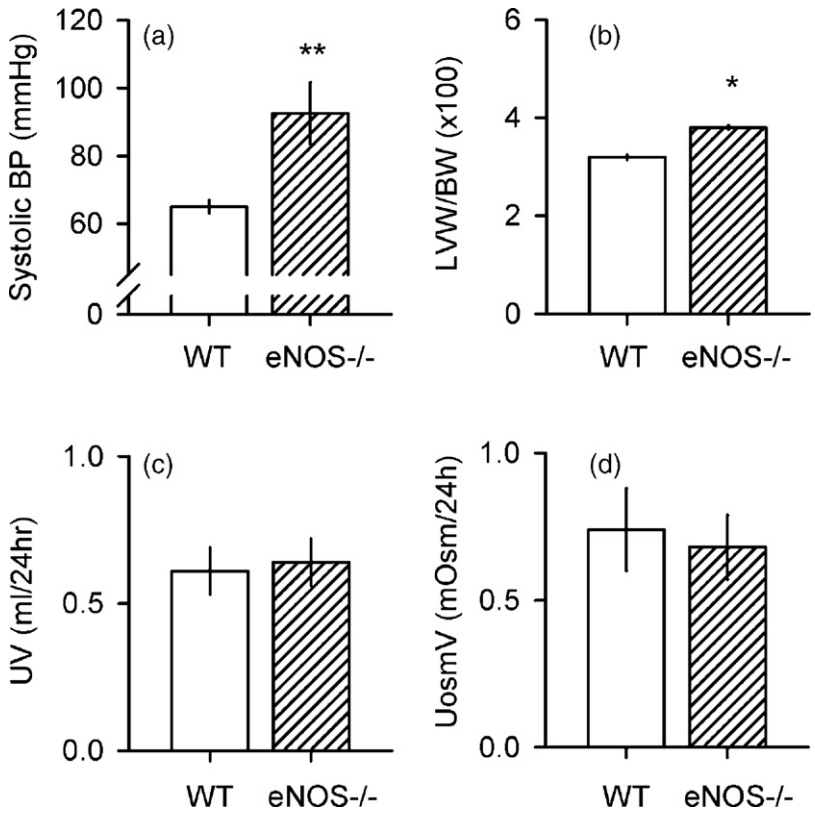

Fig. 1. Changes in systolic blood pressure, left ventricular weight, urine volume, and urinary osmolarity in eNOS−/− and control mice. Systolic BP, systolic blood pressure; LVW/BW, ratio of left ventricular weight to body weight; UV, urine volume; UosmV, urinary osmolarity; WT, wild type mice; eNOS−/−, eNOS knockout mice. $^{*}p < 0.05$; $^{**}p < 0.01$ vs wild type mice.

that disruption of the intra-renal osmotic gradient with chemical medullectomy decreases expression of NPRs mRNA and their binding capacities in renal medulla with increased cGMP content [31]. However, little information is available with regard to regulation of NPRs gene expression by eNOS and tonicity. Therefore, we evaluated the effects of eNOS expression on regulation of NPRs and tonicity-responsive enhancer binding protein (TonEBP) mRNA using eNOS-deficient mice (eNOS−/−).

2. Materials and methods

2.1. Animals

Male eNOS-deficient mice (eNOS−/−) and wild type control mice (eNOS+/+, C57B1/6J) were purchased from Jackson Laboratories (Jackson Laboratories Inc., Bar Harbor, ME). The animals were housed in individual cages in a temperature-controlled environment under a 12-h day/night cycle and fed a standard diet (5L79 Purina rat and mouse 18% chow, Charles River Laboratories Inc., Wilmington, MA) ad libitum. All experimental protocols were approved by the local animal committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

2.2. Measurements of blood pressure, urinary concentration, and tissue preparation

Mice from both strains, between 4 and 6 months of age, and weighing 20–30 g, were used for the study. Heart rate and systolic blood pressure were measured in anesthetized mice with xylazine and ketamine (1:9, 30 μl/10 g) by tail cuff plethysmography (Power Lab 2/20, AD instruments, Australia) [32]. Mice were then sacrificed, hearts were weighed, and atria and right ventricular free wall were dissected away from the left ventricle and septum [20]. The ratio of left ventricular weight to body weight (LVW/BW) was calculated and used as an index of cardiac enlargement. The left ventricles and both kidneys were frozen in liquid nitrogen until use.

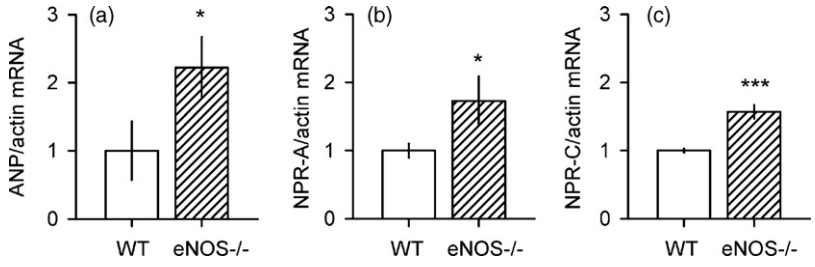

Fig. 2. Changes in expression of atrial natriuretic peptide (ANP), natriuretic peptide receptor (NPR)-A, and -C mRNA in the heart of eNOS−/− and control mice. Actin mRNA was used as a reference gene. $^{*}p < 0.05$; $^{***}p < 0.005$ vs wild type mice.