Nitric Oxide in Serum and Renal Tissue During Compensatory Renal Hypertrophy in Rats

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Abstract

Nitric oxide (NO) level in serum and renal tissue has been examined in 15 male Wistar rats, body weight 200-250 g, 7 days after unilateral nephrectomy. All rats were ether-anaesthetized and the kidneys were removed by dorsolateral approach. NO concentration in serum and renal tissue was determined by classic colorimetric Griess reaction. Conversion of NO_3^{2-} into NO_2^{2-} was done with elementary zinc. Results have shown that NO concentration in renal tissue is statistically higher in rats 7 days after unilateral nephrectomy then in control renal tissue before compensatory kidney growth ($p<0,02$). There is no difference between NO concentration in serum before unilateral nephrectomy and 7 days after nephrectomy. These findings suggest that NO may play an important role in mediating the hemodynamic changes associated with reduced renal mass.

KEY WORDS: nitric oxide, serum, renal tissue, unilateral nephrectomy.

Introduction

Nitric oxide (NO) is a highly reactive inorganic free radical, produced by many cells in the organism. NO is synthesized from L arginine by NO sinthase (NOS) which is made up of at least three isoforms (1). NO have manifold physiologic functions including modulation of vascular tone, platelet aggregation, inflammation and neurotransmission (2). At the vascular level, NO is a potent vasodilator that contributes to regulation of regional blood flow and blood pressure by tonically lowering vascular resistance (3). Reduction in renal mass after unilateral nephrectomy is associated with several structural, biochemical and functional changes in the remnant kidney (4). The hemodynamic adaptation involves increased renal blood flow (RBF) and (RVR) decreased renal vascular resistance (5). Recent studies have demonstrated that endothelium play a crucial role in the regulation of

renal vascular tone (6). NO sinthase has been identified in several epithelial cells in the kidney, including proximal tubular cells, thick ascending limb, inner medullary collecting duct, and interstitial cells (7). Previous studies have shown that NO is a major regulatory factor of RVR and RBF (8). However, a role of NO in the hemodynamic adaptation during compensatory renal growth is not clear. Valdivierso et al. (9) found that 2 days after unilateral nephrectomy, glomerular NO production was significantly higher in remaining kidney compared with controls. Thus, Sigmon et al. (10) proved that the increase in RBF after unilateral nephrectomy was blocked by inhibition of NO synthesis. Correlation between NO level in serum and renal tissue during compensatory renal growth was not studied. Thus, the aim of the present study was to investigate the effect of unilateral nephrectomy on NO level in serum and renal tissue.

Materials and Methods

1. ANIMALS

Albino Wistar rats (15 male), body weight 200-250 g, were used in experiments. All rats were offered tap water and a standard laboratory diet (14,36 KJ/g; "Sljeme", Croatia) ad libitum.

2. SURGICAL PROCEDURES

Right-side unilateral nephrectomy was performed by dorsolateral approach under diethyl-ether anesthesia as described previously (11). After 7 days period of compensatory renal growth animals were killed by cervical dislocation and the kidneys were removed immediately. All removed kidneys were cleaned from perirenal tissue, blotted, divided with transversal section into two equal parts and weighted. After heating at 105°C for 24 hours one part of kidney was weighted again. The extent of compensatory renal growth was estimated by the percent difference in absolute dry kidney mass (12).

3. SERUM SAMPLING

NO concentration in serum was measured in rats before and after unilateral nephrectomy. Blood samples for the determination of NO concentration were diluted 1:1 (vol/vol) with 0,9% saline, protein-precipitated (30% $ZnSO_4$, 0,05 ml per ml of blood), centrifuged at 2,000 g for 10 minutes and frozen at -20°C until the determination of NO level.

4. TISSUE SAMPLING

After nephrectomy, all tissue samples were weighed and washed extensively with 0,9% NaCl solution (4°C) for blood elimination. The tissue samples were placed in five time higher volume of 0,9% NaCl and homogenized in a Teflon coated Potter-Elvehjem homogenizer. After centrifugation at 4,000 g for 30 min, the supernates were protein-precipitated (30% $ZnSO_4$, 0,05 ml per ml of supernates), centrifuged and frozen at -20°C until further determination of NO level.

5. MEASUREMENT OF NO CONCENTRATION

The NO level in the blood and tissue was determined by measuring nitrite concentrations, a stable metabolic product of NO with oxygen. Conversion of NO_3^{2-} into NO_2^{2-} was done with elementary zinc. NO_2^{2-} concentration in serum and tissue was determined by classic colorimetric Griess reaction (13). Briefly, equal volumes of samples and Griess reagent were mixed at room temperature. After 5 min, the absorbance was measured at 570 nm using Perkin Elmer 550 S spectrophotometer. The concentration of nitrite was determined by a standard curve prepared with sodium nitrite (1-200).

6. STATISTICS

NO level is expressed as mean values ± SEM. Differences between the mean values were statistically compared using paired t test. Probability values of less than 0.05 were considered significant.

Results

Figure 1 shows that absolute dry kidney mass was significantly increased in rats 7 days after unilateral nephrectomy, and the mean value was by 27% higher than before compensatory kidney growth. ($p<0,001$). As shown in Figure 2. NO concentration in serum was not significantly different in rats 7 days after and before unilateral nephrectomy. Data on tissue NO level in rats before compensatory kidney growth and 7 days after unilateral nephrectomy are shown in Figure 3. NO level in the renal tissue was 20% higher in rats 7 days after unilateral nephrectomy than before compensatory kidney growth, which was statistically significant ($p<0,02$).

Discussion

It is known that the removal of a single kidney immediately stimulates the growth and function of the remaining kidney. Our study clearly showed that the mean dry kidney weight was significantly increased in rats 7 days after unilateral nephrectomy in comparison with values determined immediately after nephrectomy. Similar results were obtained in other studies (11, 12).

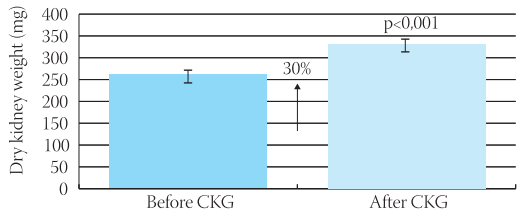

FIGURE 1. Dry kidney weight (mean±SEM) in rats (N=15) before and after unilateral nephrectomy.
Before CKG – before compensatory kidney growth
After CKG – after compensatory kidney growth

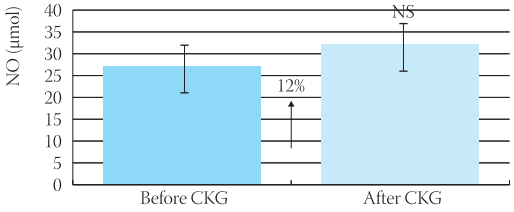

FIGURE 2. NO level in serum (mean±SEM) in rats (N=15) before and after unilateral nephrectomy.
Before CKG– data before compensatory kidney growth
After CKG – data after compensatory kidney growth
NS – not significant

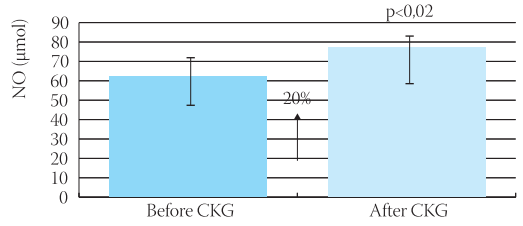

FIGURE 3. NO level in renal tissue (mean±SEM) in rats (N=15) before and after unilateral nephrectomy.
Before CKG – before compensatory kidney growth
After CKG – after compensatory kidney growth

The remnant kidney after unilateral nephrectomy undergoes compensatory growth during which there is an increase of kidney weight, RNA/DNA ratio, protein content, glomerular filtration rate and tubular reabsorption processes, leading to cell hypertrophy (14). However, very little is known about the nature of stimulus which initiates compensatory renal growth. Recent studies have suggested it is possible that NO has a role in initiation of this renal growth (9,10). NO is a potent vasodilator that contributes to regulation of regional blood flow and blood pressure by tonically lowering vascular resistance. It has been recently suggested that NO in kidneys is involved in glomerular and medullary hemodynamics (10). The hemodynamic adaptation after unilateral nephrectomy in remnant kidney includes increased renal blood flow (RBF) and decreased renal vascular resistance (RVR). However, the role of NO in compensatory kidney growth is not fully understood. Valdivielso et al. (9) have showed that NOS blockade results in a larger response of RVR in unilateral nephrectomised then in control rats. Also, NOS inhibitors induced a higher decrease in RBF in unilateral nephrectomised then in control rats. In addition, glomeruli from unilateral nephrectomised rats showed an increased NO production, which can be also blocked by NOS inhibitors. Based on these results they suggest that NO-mediated vasodilatation contributes to the adaptation of blood flow after unilateral nephrectomy. Results of this study are in agreement with that of Weisstuchy et al: (15), demonstrating that blockade of NO synthesis prevents the increase in RBF after uninefrectomy. Sigmon et al. (10) found that the immediate increase in blood flow in the remaining kidney was blocked by acute NOS inhibition. In our study, we found a significant increase of renal tissue NO level in rats after unilateral nephrectomy compared with tissue NO level in nephrectomised control kidney. However, there is not significant difference in the mean serum NO level 7 days after unilateral nephrectomy compared with values determined before compensatory kidney growth. Based on these findings it is our opinion that this increased local NO production in kidney tissue is not sufficient to cause changes of NO level in serum. Our results are in the accordance with investigation of Sigmon et al. (10) Thus, their data from bioassay of acute complete NOS inhibition suggest that the renal response was exaggerated at days 2 and 7 after unilateral nephrectomy but the systemic response was not. The mechanism of increased NO production in compensatory kidney growth is not clear. The hydromechanical forces, including pressure and shear stress, associated with pulsatile blood flow play an important role in vascular homeostasis via the control of the production and release of NO. In a circumstance of unilateral nephrectomy, hemody-

namic changes involve increased renal blood flow and vascular shear stress, which is considered to be primary stimulus for endogenous NO production from the endothelium. Continuous release of NO derived from the vascular endothelium as an important determinant of basal perfusion and resistance in the kidney is altered during compensatory kidney growth. We believe that increased local NO production after unilateral nephrectomy is responsible for local hemodynamic changes in remnant kidney. However, it is possible that this increased local NO production has no influence in systemic response on reduction of renal mass after unilateral nephrectomy. Since NO in kidneys participates in tubuloglomerular feedback, renin secretion, and extracellular fluid balance, NO synthesis and NOS expresion may be modulated by a number of factors. Further research in this area will certainly give more complete picture of NO role in compensatory kidney growth.

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