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Predisposition of spontaneously hypertensive rats to develop renal injury during nitric oxide synthase inhibition

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Abstract

Chronic nitric oxide (NO) synthase (NOS) inhibition results in renal injury. Hypertension is an important risk factor for renal injury. We studied the influence of preexistent hypertension on the sensitivity for renal injury induced by chronic NOS inhibition in rats. Spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats were treated with 3, 10, 30 and 100 mg/l *N*ω-nitro-L-arginine (L-NNA) until death. Systolic blood pressure and proteinuria were measured regularly and compared with time-control measurements in untreated SHR and WKY. In WKY, 3 and 10 mg/l L-NNA did not affect systolic blood pressure, while 30 and 100 mg/l L-NNA resulted in an increase in systolic blood pressure after 12 and 4 weeks, respectively. In contrast in SHR, every dose of L-NNA resulted in an increase in systolic blood pressure after 2 weeks. In WKY, 3 and 10 mg/l L-NNA did not affect proteinuria or survival, while 30 and 100 mg/l L-NNA resulted in an increase in proteinuria after 30 and 9 weeks, and a median survival of 36 and 12 weeks, respectively. In SHR, 3, 10, 30 and 100 mg/l L-NNA resulted in an increase in proteinuria after 30, 12, 3 and 3 weeks, and a median survival of 41, 20, 5 and 3 weeks, respectively. Thus, at every dose of the inhibitor, chronic NOS inhibition resulted in far earlier increases in systolic blood pressure and proteinuria and a marked increase in mortality in SHR as compared to WKY. Indeed, a very low dosage of L-NNA that caused no harm in WKY was followed by marked increases in proteinuria and blood pressure and decreased survival in SHR. Hypertension strongly increases the vulnerability to cardiovascular risk factors that compromise the NO-system.

Keywords: Nitric oxide (NO) synthase; Hypertension; Proteinuria; Spontaneously hypertensive rat (SHR); *N*ω-nitro-L-arginine; Survival

1. Introduction

Hypertension is one of the most important risk factors for renal failure. Approximately 30% of the incidence of end-stage renal disease in the USA is caused by hypertension (U.S. Renal Data System, 1997). Several studies in humans (Panza et al., 1993; Egashira et al., 1995) and in various animal models (Lüscher and Vanhoutte, 1986; Lüscher et al., 1987a) have shown that hypertension is associated with endothelial dysfunction. This has been attributed to decreased NO production (Lüscher et al., 1987b), increased synthesis and release of vasoconstrictors (Tschudi et al., 1994; Lüscher et al., 1987b) and products of cyclo-oxygenase (Lüscher and Vanhoutte, 1986), or increased synthesis of superoxide that inactivates NO (Tschudi et al., 1996b; Schnackenberg et al., 1998). The spontaneously hypertensive rat (SHR) is often used as a model for human hypertension (Frohlich, 1997). This model is characterized by an increased blood pressure, but the kidney is protected by increased resistance in the afferent arteriole (Dworkin and Feiner, 1986). Only aging SHR develop proteinuria (Feld et al., 1977). It has been shown that manoeuvres that stimulate the NO-pathway (Lin et al., 1997; Susic et al., 1999), as well as blockers of the angiotensin II system (Cachofeiro et al., 1995) can reduce blood pressure and improve endothelial function in SHR. This supports the concept that disturbance of the balance between vasodilators and vasoconstrictors underlies the hypertension observed in SHR.

It is conceivable that the endothelium, and particularly NO, can be regarded as a defense mechanism against renal injury induced by risk factors. Risk factors for renal injury, such as hypertension (Panza et al., 1993; Egashira et al.,

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1995; Lüscher and Vanhoutte, 1986; Lüscher et al., 1987a), diabetes (Johnstone et al., 1993) and aging (Taddei et al., 1995; Küng and Lüscher, 1995; Tschudi et al., 1996a) are all associated with a decreased availability of NO. An increased sensitivity to the deleterious effects of cardiovascular risk factors may contribute to an increased risk for renal injury. Chronic impairment of NO synthesis in normotensive rats results in hypertension and renal injury (Baylis et al., 1992; Ribeiro et al., 1992; Verhagen et al., 1998). However, whether SHR are more sensitive to the development of renal injury during chronic NOS inhibition than WKY is unknown. Chronic NOS inhibition in SHR with *N*-nitro-L-arginine methyl ester (L-NAME) during 3 weeks resulted in severe renal injury with marked proteinuria. However, only one high dose of L-NAME was used and WKY rats were not studied (Ono et al., 1995). In the present study, chronic NOS inhibition was regarded as a universal risk factor and proteinuria was used as a marker of renal injury. We hypothesized that chronic NOS inhibition, with low doses of the NOS inhibitor *N*ω-nitro-L-arginine (L-NNA) that would not harm normotensive WKY rats, would induce proteinuria in SHR. Rats were treated with different concentrations of L-NNA until death to obtain survival curves.

2. Materials and methods

2.1. Animals

Male SHR and WKY rats, weighing 125–150 g at start protocol, were purchased from Iffa Credo (France), and housed under conventional conditions, receiving a standard natural diet (RMH-TM: protein 22.2%, fat 4.8%, potassium 0.85%, sodium 0.4%; Hope Farms, Woerden, The Netherlands). Sentinel animals, which were monitored regularly for infection by nematodes and pathogenic bacteria, and antibodies to many rodent viral pathogens (International Council for Laboratory Animal Science, Nijmegen, The Netherlands), tested negative for infection throughout the experiment. The protocol was approved by the Utrecht University Board for studies in experimental animals.

2.2. Protocol

SHR and WKY rats received the NOS inhibitor L-NNA (Sigma, USA) dissolved in drinking water at zero (con), 3, 10, 30 and 100 mg/l until death ($n = 6–8$ rats per strain per dose). Systolic blood pressure was measured in the awake rats by the tail-cuff method (IITC, San Diego, USA). The rats were placed in metabolism cages for 24 h for determination of urinary protein excretion. Urinary protein concentrations were determined by the Bradford method (Bradford, 1976). Systolic blood pressure and proteinuria were measured approximately once every 2 weeks in the SHR receiving 30 and 100 mg/l L-NNA and the WKY receiving 100 mg/l L-NNA and once every 6 weeks in the other rats until death in all treated SHR (61 weeks). The remaining rats (SHR con, WKY con, WKY 3 mg/l and WKY 10 mg/l L-NNA) were followed until death to obtain survival data.

2.3. Statistical methods

Systolic blood pressure and proteinuria are expressed as mean ± S.E.M. It was tested when treatment of WKY and SHR with the different concentrations L-NNA resulted in a significant increase in systolic blood pressure or proteinuria when compared with time-control measurements in untreated WKY and SHR by one-way analysis of variance followed by Dunnett's test. Survival is presented as median (range). Comparisons between survival in WKY and SHR treated with the same concentration L-NNA were done by Mann–Whitney Rank Sum Test.

3. Results

3.1. Systolic blood pressure

In WKY, treatment with the lowest concentrations of L-NNA (3 and 10 mg/l) did not result in a significant increase in systolic blood pressure, although systolic blood pressure in WKY treated with 10 mg/l was numerically higher than control after 18 weeks of treatment (Fig. 1). Treatment of WKY with 30 and 100 mg/l L-NNA resulted in a significant increase ($P < 0.05$) in systolic blood pressure after 12 and 4 weeks, respectively. In contrast, in SHR every dose of L-NNA resulted in an immediate increase in systolic blood pressure that was significantly different from control SHR ($P < 0.05$) starting from the first measurement, namely at 2 weeks (Fig. 1). In SHR treated with the lowest concentration L-NNA, 3 mg/l, systolic blood pressure stayed numerically higher than in control SHR until 42 weeks of treatment, although this was not significant at every time point. Thus, systolic blood pressure was increased by L-NNA far earlier at every dose in SHR compared with WKY ($P < 0.05$; Table 1).

3.2. Proteinuria

In untreated SHR, proteinuria was slightly higher than in untreated WKY, but this difference did not increase in the first 48 weeks of the study (Fig. 2). An increase in proteinuria was always preceded by an increase in systolic

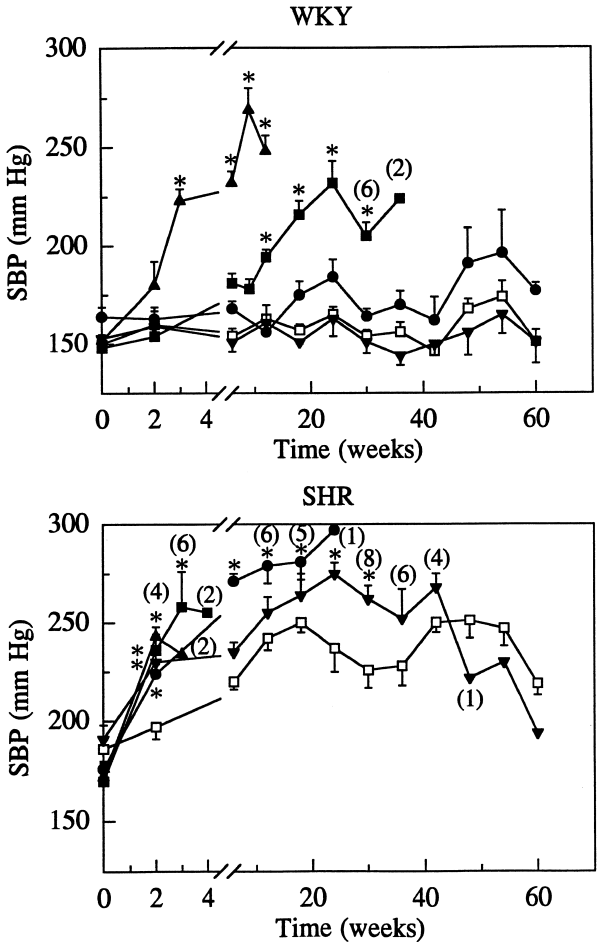

Fig. 1. Systolic blood pressure in WKY and SHR treated with 0, 3, 10, 30 and 100 mg/l *N*ω-nitro-L-arginine until death. □: control; ▼: (3 mg/l); ●: (10 mg/l); ■ (30 mg/l); ▲: (100 mg/l). $^{*}P < 0.05$ vs. control. When rats died the new (*n*) is shown; the first (*n*) shows the original number of rats.

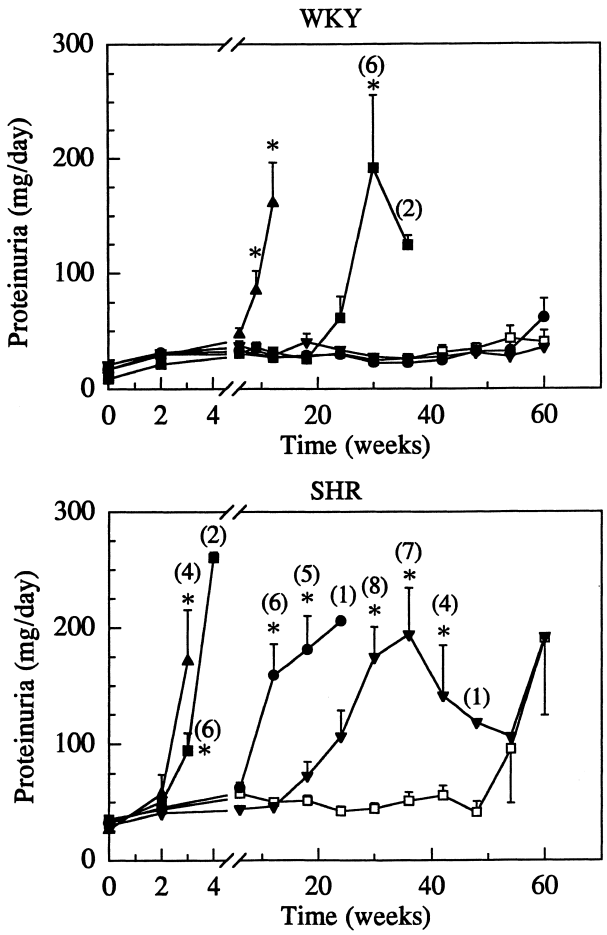

Fig. 2. Proteinuria in WKY and SHR treated with 0, 3, 10, 30 and 100 mg/l *N*ω-nitro-L-arginine until death. Symbols as in Fig. 1.

blood pressure. In WKY, treatment with the lowest doses of L-NNA (3 and 10 mg/l) did not result in a significant increase in proteinuria. Treatment of WKY with 30 and 100 mg/l L-NNA resulted in a significant increase ($P < 0.05$) in proteinuria after 30 and 9 weeks, respectively. In SHR, treatment with 3, 10, 30 and 100 mg/l L-NNA resulted in a significant increase ($P < 0.05$) in proteinuria after 30, 12, 3 and 3 weeks, respectively (Fig. 2). Thus, proteinuria was increased far earlier at every dose of L-NNA in SHR compared with WKY ($P < 0.05$; Table 1).

Table 1
Start hypertension, start proteinuria and median survival in SHR and WKY treated with 3, 10, 30 and 100 mg/l *N*ω-nitro-L-arginine (L-NNA)

L-NNA (mg/l)	3	10	30	100
Start hypertension WKY (week)	–	–	12	4
Start hypertension SHR (week)	2	2	2	2
Start proteinuria WKY (week)	–	–	30	9
Start proteinuria SHR (week)	30	12	3	3
Median survival WKY (week)	111	87	36	12
Median survival SHR (week)	41	20	5	3

Start hypertension and proteinuria was defined as the first week when treatment with L-NNA resulted in a significant increase in blood pressure and proteinuria, respectively, when compared to control.

3.3. Survival

Survival in WKY treated with 3 and 10 mg/l L-NNA was not different from control (Fig. 3). Treatment of WKY with 30 and 100 mg/l L-NNA resulted in an increase in mortality rates when compared to control. Median survival was 36 weeks (range: 30–39 weeks) in WKY treated with 30 mg/l L-NNA and 12 weeks (11–15 weeks) in WKY treated with 100 mg/l L-NNA. In contrast in SHR, every dose of L-NNA resulted in an increased mortality rate when compared to control. Treatment of SHR with 3, 10, 30 and 100 mg/l L-NNA resulted in a median survival of 41 (29–61) weeks, 20 (15–27) weeks, 5 (4–5) weeks and 3 (2–4) weeks, respectively (Fig. 3). Thus, mortality was

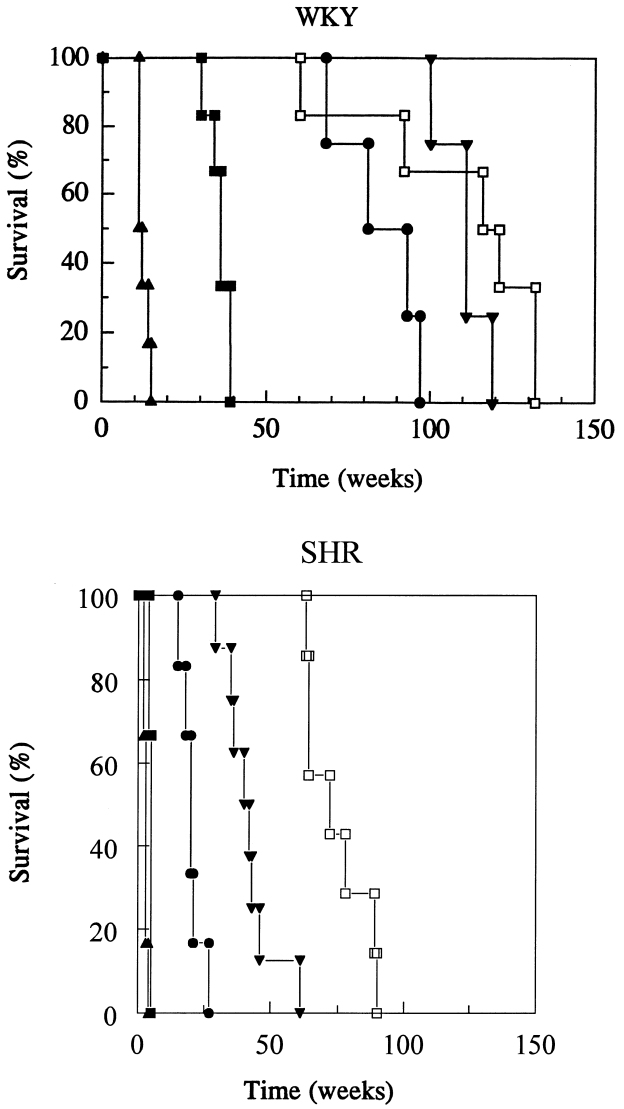

Fig. 3. Survival in WKY and SHR treated with 0, 3, 10, 30 and 100 mg/l *N*ω-nitro-L-arginine. Symbols as in Fig. 1.

increased at every dose of L-NNA in SHR compared with WKY ($P < 0.05$; Table 1).

4. Discussion

We investigated whether an increased sensitivity to impairment of NO synthesis contributes to the increased risk for renal injury associated with hypertension. To make an accurate estimate of the influence of hypertension on the sensitivity to chronic NOS inhibition, SHR and WKY were treated with four concentrations of L-NNA until death. This study shows that chronic NOS inhibition resulted in a far earlier increase in proteinuria and a marked increase in mortality in SHR as compared with WKY, at every concentration of L-NNA. Indeed, 10 mg/l L-NNA, a very low dosage that caused no harm in WKY, was followed by marked increases in proteinuria and systolic blood pressure and decreased survival in SHR. Thus, hypertension strongly increases the vulnerability to cardiovascular risk factors that interfere with the NO-system.

This study shows for the first time that hypertension increases the sensitivity for the effects of chronic NOS inhibition on renal injury. It has been shown that treatment of SHR with L-NAME resulted in increased afferent and efferent glomerular arteriolar resistance as compared to control SHR, and severe afferent fibrinoid necrosis, segmental glomerular hyalinosis and sclerosis and renal ischemia with marked proteinuria (Ono et al., 1995, 1996a, b; Francischetti et al., 1998; Nakamura et al., 1999; Qiu et al., 1999). However, the effects of L-NAME in WKY were not studied in any of these studies. The present study shows that an increase in proteinuria was always preceded by an increase in systolic blood pressure, suggesting that increased blood pressure contributed to the development of proteinuria. The mechanism relating increased blood pressure to proteinuria in this model is unknown. Although, glomerular hypertension has been observed after chronic NOS inhibition in normotensive rats (Baylis et al., 1992), no glomerular hypertension was observed after chronic NOS inhibition in SHR (Ono et al., 1995). Alternatively, glomerular permeability may have been increased, because chronic NOS inhibition increases endothelial permeability (Kubes and Granger, 1992; Cordona Sanclemente and Born, 1995).

Why does hypertension increase the sensitivity to chronic NOS inhibition? In the SHR, there is not a deficiency of NO. Most reports show that in SHR, protein expression of endothelial NOS (eNOS) and eNOS activity are upregulated in a variety of organs, including kidney (Hayakawa and Raij, 1998; Vaziri et al., 1998; Nava et al., 1996), aorta (Hayakawa and Raij, 1998; Vaziri et al., 1998), heart (Nava et al., 1996) and mesenteric resistance vessels (Nava et al., 1996), when compared with WKY. Urinary excretion and plasma concentration of NO metabolites have been reported to be increased in SHR (Vaziri et al., 1998; Nava et al., 1996). However, this overproduction of NO did not result in increased formation of cGMP in SHR as compared with WKY, since similar cGMP levels have been reported in the aorta (Arnal et al., 1993), heart (Nava et al., 1996) and mesenteric resistance vessels (Nava et al., 1996). A possible explanation is that in SHR part of the produced NO is inactivated by oxygen radicals. Indeed, acute injection of a fusion protein containing human Cu/Zn-type superoxide dismutase (Nakazono et al., 1991), and acute and chronic treatment with tempol, a superoxide dismutase mimic, decreased blood pressure significantly in SHR, but did not affect blood pressure in WKY (Schnackenberg et al., 1998). Hypertension in SHR has also been associated with increased production of vasoconstrictors (Lüscher and Vanhoutte, 1986; Tschudi et al., 1994; Cachofeiro et al., 1995). The imbalance between vasodilators and vasoconstrictors

that underlie hypertension may explain the increased sensitivity to manoeuvres that further interfere with this balance, such as NOS inhibition.

Treatment of SHR with the lowest concentration of L-NNA (3 mg/l) and treatment of WKY with 30 mg/l L-NNA only resulted in an increase in proteinuria after 30 weeks of treatment. Aging kidneys rely more on NO-related mechanisms (Reckelhoff and Manning, 1993; Hill et al., 1997). This may be due to a decreased production of NO, since decreased immunostaining for eNOS in renal peritubular capillaries (Thomas et al., 1998) and decreased urinary nitrate/nitrite excretion were observed with aging in normotensive (Reckelhoff and Manning, 1993; Reckelhoff et al., 1997) and also hypertensive rats (Maeso et al., 1998). In addition, free oxygen radicals play an important role in aging. Higher formation rates of free radicals, accumulation of free radical damage and changes of antioxidant capacities have all been reported in aging animals (Nohl, 1993). This suggests that aging in general results in a loss of renal availability of NO, which may increase sensitivity to NOS inhibition.

The present study shows that even low grade NOS inhibition resulted in renal damage in the presence of hypertension. Thus, hypertension is not only a risk factor for renal failure, but it also increases the sensitivity to renal injury induced by chronic NOS inhibition. This suggests that cardiovascular risk factors that compromise the NO-system, such as diabetes and aging, are more detrimental in the presence of hypertension. In conclusion, the variable end-organ disease observed in hypertensive individuals may be explained in part by the presence of other risk factors that compromise the NO-system.

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