Strain Differences of Hypertension Induced by Dietary N^G-nitro-L-arginine in Normotensive Rats

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Abstract: When the potent inhibitor of nitric oxide (NO) synthesis N^G-nitro-L-arginine (L-NNA) was incorporated into the diet, hypertension was induced and sustained due to the effects of the long-term inhibition of endothelium-dependent relaxing factor (EDRF)/NO. The effects of L-NNA on normotensive rats of four strains (Donryu, Sprague-Dawley (SD), Wistar, and Wistar-Kyoto (WKY)) were compared relative to control rats. L-NNA administration caused a sharp initial increase in systolic blood pressure (SBP) at 2 weeks in all animals, and this was followed by a gradual and steady increase until 4 weeks. At the end of the experiments (5 weeks), the mean SBP of Donryu and SD rats was decreased. The maximum blood pressure of Donryu and Wistar rats during the experiments exceeded 200 mmHg, but that of SD and WKY rats was below 200 mmHg. Body weight loss and death were observed only in L-NNA-fed Donryu rats. Pathological changes in the kidneys and the morbidity rates for the lesions were determined, and indicated that the Donryu L-NNA group was 100% positive. These results suggest that the Donryu strain is more sensitive to L-NNA than the other strains. That dietary L-NNA-induced hypertension in normotensive rats of the four strains provides a new artificially-induced hypertensive model in which vasoconstriction occurs mainly due to EDRF deficiency.

Key words: EDRF, experimental hypertension, L-NNA, rats, strain differences

Introduction

In 1980, Furchgott and Zawadzki demonstrated that vascular relaxation induced by acetylcholine was dependent on the presence of functionally intact endothelium, and postulated the release of endothelium-dependent relaxing factor (EDRF) [5]. Since then, the identification of EDRF has remained controversial, but nitric oxide (NO) has been demonstrated to be the ma-

jor factor affecting the biological activities of EDRF [12]. Later, N^G-nitro-L-arginine (L-NNA) was proven to inhibit the endothelium-dependent relaxation and the synthesis of NO [17, 21, 22]. Gardiner *et al.* [6] orally administered N^G-nitro-L-arginine methyl ester (L-NAME) to vasopressin-deficient Brattleboro rats for several hours and observed increased mean blood pressure. Ikeda *et al.* [13, 14] reported that the prolonged ingestion of dietary L-NNA could induce sustained hy-

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pertension in normotensive male Wistar-Kyoto rats (WKY).

In the present study, we investigated whether strain differences in reaction to dietary L-NNA could induce sustained hypertension in four strains of normotensive rats, because these strains were commonly used for animal experiments.

Materials and Methods

Animals: A total of 48 male rats, 12 of each strain, were used. All experiments were performed according to the animal experiment guidelines of Shimane Medical University. The rat strains used were HOS:Donryu (Donryu) and Slc:Sprague-Dawley (SD) from SLC Inc., Shizuoka, Jcl:Wistar (Wistar) from CLEA Japan Inc., Tokyo, and WKY/NCrj (WKY) from Charles River Japan Inc., Kanagawa. At the age of 7 weeks, they were purchased from the breeder, acclimatized to their new environment for one week before the experiment, and used at 8 weeks of age.

Experimental design: Each strain was divided into the following 2 groups: 0.023% (w/w) L-NNA (Peptide Institute Minoo, Osaka)-fed group and control group fed a plain commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo) for a period of 5 weeks ($n=6$ rats/group, respectively). Food and water were available *ad libitum*. The temperature, relative humidity and light/dark cycle were maintained at $23 \pm 2^\circ\text{C}$, $55 \pm 10\%$, 12 hr light/dark cycle (8 a.m.–8 p.m.), respectively. Systolic blood pressure (SBP) was measured by the same method as previously reported with a photoelectric oscillometer [15]. Briefly, a rat was first warmed in a hot box at 38°C for 10 min, and then placed in a restraining apparatus which was also kept at 38°C . The tail was inserted through the cuff (Size: 12 mm $\varnothing$, Standard) which contained a photoelectric pulse detector, and SBP was recorded when the first oscillation appeared during gradual reduction of the cuff pressure. Body weight and SBP were measured twice a week for 2 weeks, and once a week thereafter. Food consumption, water consumption and urine volume were measured over a 24-hr period every 2 weeks with a metabolic cage. The amounts of Na and K were measured by ion-selective electrode methods (SE-1510; Kyoto Daiichi Kagaku Co., Ltd., Kyoto). All the rats were fasted and given only *tap water* to drink for 24-hr before death. They

were anesthetized with sodium pentobarbital (50 mg/kg), killed by heart puncture, and the brain, heart and kidneys were removed.

Histology: The kidneys obtained from each rat were fixed in 10% neutral buffered formalin, and paraffin sections (4 μm) were stained with hematoxylin and eosin (HE) and periodic acid - Schiff (PAS) reaction. Grading of histological changes: the overall severity of arteriolar wall thickening and glomerular lesions including pigmentation were evaluated from - (negligible) to + (marked).

Statistical analyses: Data are expressed as the mean $\pm$ standard error mean (SEM). Statistical analyses were performed by employing the unpaired Student's *t*-test, and a *p*-value less than 0.05 was considered to be significant.

Results

Survival rate: The survival rate at the end of the 5-week study (13 weeks of age) in SD, Wistar and WKY strains fed on L-NNA diet was 100%. However, that of Donryu rats fed L-NNA diet was 83.3% at the end of the 5 week-study, and one death occurred at 3 weeks.

Body weight: Until 3 weeks, all rats grew linearly, and there were no major differences in the growth compared with control groups. The Wistar strain showed slight body weight loss at 5 weeks, but it was not significant, but the mean body weight of the Donryu L-NNA group was significantly decreased: about 52 g at 4 weeks and 121 g at 5 weeks (Fig. 1).

Blood pressure: As shown in Fig. 2, prolonged oral administration of dietary L-NNA in the four strains of rat induced sustained hypertension. In the control group of Donryu rats, SBP values ranged from 160 to 170 mmHg. The Donryu L-NNA group had a decrease in the first 3 days (from 166 ± 3 mmHg to 144 ± 4 mmHg), and developed severe hypertension (over 200 mmHg) from 2 weeks. The maximum SBP value was 230 ± 9 mmHg (at 4 weeks) but had decreased at 5 weeks to 205 ± 11 mmHg. In the control group of SD rats, SBP values were in the normal range (below 150 mmHg). The L-NNA group of SD rats showed a sharp increase in hypertension during the first 3 days (from 135 ± 4 mmHg to 161 ± 3 mmHg), a steady and sustained increase up to 4 weeks, and a slight decrease at 5 weeks. The maximum SBP value was 191 ± 6 mmHg (at 4

Fig. 1. Body weight of four strains of rat fed an L-NNA-supplemented diet or a control diet for a period of 5 weeks. Data are presented as mean $\pm$ SEM. Significant differences from control, *: $p < 0.05$, **: $p < 0.01$. (): Number of rats examined; the other groups included 6 animals are not shown.

weeks). In the Wistar control group, SBP values ranged from 140 to 155 mmHg. The Wistar L-NNA group showed a sharp increase in hypertension during the first 3 days (from 137 ± 4 mmHg to 172 ± 3 mmHg), and a steady and sustained increase up to 5 weeks. The maximum SBP value was 233 ± 7 mmHg at 5 weeks. In the WKY control group, SBP values ranged from 127 to 140 mmHg. The WKY L-NNA group showed a sharp increase in blood pressure at 1 and 5 weeks, to 151 ± 4 and 198 ± 6 mmHg, respectively. A significant rise in SBP was observed in the first 3 days in SD and Wistar

L-NNA groups.

Food consumption (g/day): As shown in Fig. 3, the Donryu L-NNA group had decreases in food consumption at 1, 3 and 5 weeks, to 16 ± 4 , 15 ± 4 and 4 ± 2 g/day, respectively, and significantly lower consumption ($p < 0.01$) than the control group at 5 weeks. In the SD, there were no significant differences between food consumption in the L-NNA and control groups throughout the experiment period. Food consumption in the Wistar L-NNA group was significantly lower ($p < 0.05$) than in the control group at 5 weeks. Consumption in the WKY

Fig. 2. Systolic blood pressure of four strains of rat fed an L-NNA-supplemented diet or a control diet for a period of 5 weeks. Data are presented as mean $\pm$ SEM. Significant differences from control, *: $p < 0.05$, **: $p < 0.01$. (): Number of rats examined; the other groups included 6 animals.

L-NNA group was significantly lower ($p < 0.01$) than in the control group at 3 weeks, so that the Donryu L-NNA group had the lowest food consumption among the four strains.

Urine volume (ml/day): As shown in Fig. 4, the urine volume of Donryu rats was significantly lower ($p < 0.05$) in the L-NNA group than in the control group at 1 week; thereafter, that of the L-NNA group was slightly high at 5 weeks. The urine volume of SD rats was significantly lower ($p < 0.05$) in the L-NNA group than in the control group at 5 weeks. The urine volume of

Wistar rats was significantly higher ($p < 0.01$) in the L-NNA group than in the control group at 3 weeks. WKY fed L-NNA had a significant greater urine volume than the control group at 1, 3 and 5 weeks. The urine volume had a tendency to increase in the L-NNA groups of Wistar and WKY rats throughout the experiment period.

Urinary Na and K excretion (mmol/day): As shown in Fig. 5. Urinary Na concentration in the Donryu L-NNA group was significantly lower ($p < 0.05$) than in the control group at 1 week. The urinary Na concen-

Fig. 3. Food consumption of four strains of rat fed an L-NNA-supplemented diet or a control diet. Data are presented as mean $\pm$ SEM. Significant differences from control, *: $p < 0.05$, **: $p < 0.01$. (): Number of rats examined; the other groups included 6 animals.

trations of the L-NNA-fed SD, Wistar and WKY rats were not significantly different from those of the control groups. The urinary K concentration of the Donryu rats was significantly lower in the L-NNA group than in the control group at 1 and 5 weeks. The other three strains showed no differences in urinary K concentration. There was no significant difference in the urinary Na/K ratio between the L-NNA group and control group of the four strains.

Organ weight: In the Donryu rats, weights of the brain and kidneys were significantly smaller ($p < 0.05$) in the L-NNA group than in the control group. In the

SD rats, the brain was significantly heavier ($p < 0.05$) in the L-NNA group than in the control group. In the Wistar rats, the heart weight was significantly greater ($p < 0.01$) in the L-NNA group than in the control group. WKY rats showed no differences between the L-NNA and control groups in organ weights (Table 1).

Pathological changes in kidneys and the morbidity rate of the lesions: Administration of L-NNA was associated with widespread renal morphological changes, i.e. marked thickening of the wall of small arteries and arterioles, frequently in association with the collapse of the glomerular tuft. Especially in the Donryu L-NNA

Fig. 4. Urine volume of four strains of rat fed an L-NNA-supplemented diet or a control diet. Data are presented as mean $\pm$ SEM. Significant differences from control, *: $p < 0.05$, **: $p < 0.01$. (): Number of rats examined; the other groups included 6 animals.

group, there was hyaline sclerosis of the glomerulus, and arteriolar contraction and medial thickening (Fig. 6). Differences in the morbidity rates of kidneys were observed between the L-NNA group and control group among the four strains (Table 2). The morbidity rate of the Donryu L-NNA group was 100% positive for nephrosclerosis in the kidneys. That of the Wistar L-NNA group was 83%, and those of the SD and WKY groups were 67 and 33%, respectively.

Discussion

This study clearly demonstrated that the administration of L-NNA in the diet induced sustained hypertension in four strains of normotensive rats. These results agreed with the findings of Ikeda *et al.* [13, 14]. They showed that the effect of dietary L-NNA on SBP was characterized by steady and sustained increase until 5 weeks, but the effect on the blood pressure of WKY rats in the present experiments differed from their results [13] for the WKY strain. Nabika *et al.* [23] and Kurtz *et al.* [18] reported substantial genetic differences

Fig. 5. 24 hr urinary Na and K excretion of four strains of rat fed an L-NNA-supplemented diet or a control diet. Data are presented as mean $\pm$ SEM. Significant differences from control, *: $p < 0.05$, **: $p < 0.01$. (): Number of rats examined; the other groups included 6 animals.○....: Control Na, —●—: L-NNA Na,□....: Control K, —■—: L-NNA K.

between the WKY/NCrj strain and the WKY/Izm strain as judged by DNA fingerprinting patterns. Ikeda *et al.* used the WKY/Izm strain and we used WKY/NCrj strain, and we therefore suppose that the differences in the effects on blood pressure in WKY rats may have resulted from differences between these strains.

L-NNA is a guanidonitro arginine derivative that impairs endothelium-dependent dilation by inhibiting cytosolic NO synthesis [22]. Previously it was reported that NO was synthesized from L-arginine in endothelial cells [25]. L-NNA and other NO synthesis inhibitors cause a rise in blood pressure in many species, including rats [16, 27], guinea pigs [1, 30], rabbits [21, 22,

26] dogs [31] and mice [11]. Inhibition of NO synthesis leads to long-lasting, but reversible, hypertension [4, 7, 24].

In this experiment, L-NNA administration caused a sharp initial increase in SBP until 2 weeks in all the rats. Furthermore, it should be noted that at the end of 5 weeks of study, the blood pressure of Donryu and Wistar rats exceeded 200 mmHg, but that of SD and WKY rats did not. When we compared the maximum SBP value in the L-NNA group with that in the control group, Donryu and Wistar rats had an SBP increase ranging from 70 mmHg to 80 mmHg, whereas SD and WKY rats had an increase ranging from 40 mmHg to

Table 1. Organ weights of four strains

Strain	Groups	Number of rats	Brain (g)	Heart (g)	Kidneys (g)
Donryu	Control	6	2.126 ± 0.019	1.479 ± 0.090	2.869 ± 0.113
	L-NNA	5	2.020 ± 0.040*	1.497 ± 0.060	2.337 ± 0.130*
SD	Control	6	1.962 ± 0.033	1.213 ± 0.039	2.317 ± 0.062
	L-NNA	6	2.082 ± 0.025*	1.207 ± 0.041	2.226 ± 0.056
Wistar	Control	6	1.846 ± 0.015	1.062 ± 0.022	2.136 ± 0.010
	L-NNA	6	1.842 ± 0.041	1.255 ± 0.052**	2.041 ± 0.115
WKY	Control	6	2.338 ± 0.019	1.253 ± 0.037	2.476 ± 0.047
	L-NNA	6	2.319 ± 0.012	1.300 ± 0.026	2.585 ± 0.044

Each value indicates the mean ± SEM. Significant difference from control, *: P<0.05, **: P<0.01.

Table 2. The morbidity rate of the lesions observed in kidneys of four strains of rats fed on L-NNA diet and control diet

Strain	Groups	Number of rats	Positive for nephrosclerosis ^a	
			grade + (%)	grade - (%)
Donryu	Control	6	0 ^b	6 (100)
	L-NNA	5	5 (100)	0
SD	Control	6	0	6 (100)
	L-NNA	6	4 (67)	2 (33)
Wistar	Control	6	0	6 (100)
	L-NNA	6	5 (83)	1 (17)
WKY	Control	6	0	6 (100)
	L-NNA	6	2 (33)	4 (67)

a: renal histology revealed angioneclerosis (fibrinoid necrosis) in afferent arteriols and small arteries. b: number exhibiting corresponding lesions.

60 mmHg, so that the tendency for SBP to increase showed differences between these two groups. These differences suggest that Donryu and Wistar rats are more dependent on basal NO production in vasodilation to maintain normal blood pressure. Especially only Donryu rats showed a temporary decrease in SBP in response to L-NNA in the first 3 days of this study. The reason for this early SBP decrease is not known, but an SBP decrease means that the vasodilation occurred in the first 3 days. The abolition of the effect of dietary L-NNA on blood pressure by L-arginine HCl infusion has already been described [9, 13]. One possibility is that Donryu rats are able to produce NO even within 3 days after L-NNA administration because of a large amount of pooled L-arginine in the body. Another possibility is that Donryu rats may have excess induction of interferon- γ (IFN- γ) [8] or tumor necrosis factor- α (TNF- α) [20] to further increase the expres-

Fig. 6. Photomicrograph shows hyaline sclerosis of glomerulus (arrow) and medial thickening (arrowhead) in renal arteries of L-NNA-treated Donryu rats. HE staining; magnificant, × 250.

sion of NO synthesis with supposed inducible NO synthase (i-NOS). Unfortunately we did not measure the SBP at 1 and 2 days after L-NNA administration. In these early days the other 3 strain rats might also have an SBP decrease, and further experiments should be done. One other thing, Donryu rats had an increase in fundamental blood pressure. This suggests that the response of vascular smooth muscle to NO was also partially attenuated, in relation to the possibility that a temporary decrease in SBP due to a functional abnormality to produce NO for some reason or other.

Measurement of body weight and food consumption are useful methods for early detection of the onset of disease or debilitation in animals. Body weight and food consumption of the Donryu L-NNA group were greatly decreased at 5 weeks, and the Wistar L-NNA

group had a slight body weight loss and decrease in food consumption at 5 weeks. The Donryu and Wistar rats were therefore more sensitive to L-NNA than the other strains. This sensitivity of the Donryu strain was accompanied by noticeable body weight loss and one death.

Earlier reports [3, 19, 28, 29] suggest that chronic inhibition of NO synthase (NOS) induced by L-NNA increases kidney functional disorders. Alberola *et al.* [2] observed that a deficiency in NOS might constitute an important factor in the development of systemic hypertension, because such a deficiency interferes with the ability of the kidneys to excrete sodium and water. The finding in the present experiments of reduced excretion of Na and reduced urine volume at 1 and 3 weeks in the Donryu L-NNA group are thought to be related to these deficiencies in NOS. The other three strains did not clearly show the urinary changes, but the reason for this difference is unclear. Changes in urine production may be related to kidney functional disorders. In particular, the Donryu L-NNA group had a 100% morbidity rate for nephrosclerosis of the kidneys. The Wistar L-NNA group had an 83% nephrosclerosis morbidity rate. Arterial hypertension was associated in our experiments with marked renal histological changes. In addition, changes such as hyaline sclerosis and angioneclerosis (fibrinoid necrosis) in afferent arteriols and small arteries, and wall-thickening of those vessels with marked folding of internal elastic layers and inward bulging of endothelial cells, are suggestive of severe vasoconstriction occurring in the L-NNA groups [27, 32]. Additionally, segmental glomerular lesions were frequently observed in the L-NNA groups of Donryu and Wistar rats. Of further interest is the tendency to a heart weight increase in the Wistar L-NNA group observed in this study. These results suggest the possibility of microvascular lesions characteristic of hypertension, such as extreme arteriolar [10] and wall thickening hypertrophy [33] in the Wistar L-NNA group.

In recent investigations on EDRF, Ikeda *et al.* [13, 14] reported dietary L-NNA-induced hypertension in normotensive WKY/Izm rats, as a new artificially-induced hypertensive model in which vasoconstriction occurred mainly due to EDRF deficiency. The present results in four normotensive strain studies as well as the other studies reported [13, 27], may be used in

establishing a new artificially-induced hypertensive model.

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