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# Ventricular hypertrophy and arterial hemodynamics following deprivation of nitric oxide in rats

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**Abstract**

In the present study, we elucidated the possible role of hemodynamic parameters and chemical factors in the development of ventricular hypertrophy (VH) following chronic nitric oxide (NO) deprivation with $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME). Impedance spectral analysis was used to obtain the arterial hemodynamics including the steady and pulsatile components. Body weight (BW), left ventricular (LV) weight (LVW), LVW/BW ratio, LV collagen volume fraction (LVCVF), cyclic GMP, and nitrite/nitrate were measured. The extent of VH was evaluated by the LW/BW, total number, numerical density, and size of cardiomyocytes. Sprague–Dawley rats were given L-NAME 10, 20, and 40 mg/kg/day from the age of 10 to 18 weeks. Control and age-matched rats were given vehicle for the same period. Treatment of L-NAME for 8 weeks caused a dose-dependent increase in tail cuff pressure and a reduction in BW with increases in LVW, LVW/BW, number, numerical density, and size of myocytes. There was elevation of aortic pressure with decreases in cardiac output, and arterial compliance. The total peripheral resistance, characteristic impedance and pulse wave reflection were increased. Histological finding revealed severe myocardial hypertrophy and fibrosis with fibroblast infiltration. The LVCVF was increased, while LV cGMP and nitrite/nitrate were reduced in a dose-dependent manner. The results suggest that chronic NOS blockade causes hypertension, impairment of large vessel properties, and VH. The development of VH may result partly from the decreases in cGMP and nitrite/nitrate in the ventricle. Correlation analysis indicates that the extent of VH is equally related to the steady and pulsatile hemodynamics.


*Keywords:* Hemodynamics; Hypertension; Ventricular hypertrophy; Total peripheral resistance; Impedance; Nitric oxide synthase

## Introduction

Nitric oxide (NO) released from endothelial cells has been shown to account for the biological properties of endothelium-derived relaxing factor (EDRF) (Moncada et al., 1991; Palmer et al., 1988). The formation of NO from L-arginine through the action of NO synthase (NOS) activates guanylate cyclase and leads to an increase in cyclic guanosine monophosphate (cGMP), which in turn causes relaxation in vascular smooth muscle (Palmer et al., 1988; Yamazaki et al., 1991). Accordingly, the continuous release of endogenous NO maintains a dilator tone in vascular tissues (Fitch et al., 2001; Rees et al., 1989; Zanchi et al., 1995). Previous studies have demonstrated that in isolated aortic rings, perfused vascular beds, and whole animals, acute blockade of NO release causes vasoconstriction (Chang et al., 2002; Ward and Angus, 1993; Zanchi et al., 1995). In addition, NOS inhibition inevitably produces increases in total peripheral resistance and arterial pressure in the whole body (Arnal et al., 1992, 1993; Baylis et al., 1992; Chen and Hu, 1997; Hu et al., 1994a, 1997; Manning et al., 1993; Rees et al., 1989). There is thus little doubt that endothelium-derived NO plays a pivotal role in the regulation of arterial pressure and hemodynamics (Arnal et al., 1992; Baylis et al., 1992; Chen and Hu, 1997; Hu et al., 1994a, 1997; Manning et al., 1993; Moncada et al., 1991).

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In addition to the studies on the acute effects of NOS blockade, previous experiments in animals have suggested that a chronic NOS blockade with several L-arginine analogues causes sustained hypertension (Arnal et al., 1992, 1993; Baylis et al., 1992; Morton et al., 1993). Animal experiments conducted by Arnal et al. (1992, 1993) revealed in rats that long-term (8 weeks) administration of $N^{\omega}$-nitro-arginine methyl ester (L-NAME) significantly reduced the aorta cyclic guanosine monophosphate (cGMP) and elevated the arterial pressure, but did not change the left ventricular weight (LVW) and the LVW to body weight (BW) ratio. The absence of ventricular hypertrophy (VH) contrasted with results obtained in other models of experimental hypertension, in which long-term hypertension was accompanied by overt VH in essential, spontaneous, renovascular and DOCA-salt hypertension or hypertensive disease of primary aldosteronism (Bianciotti and De Bold, 2002; Ehamke et al., 1999; Hu et al., 1994b; Morton et al., 1993; Suzuki et al., 1988; Yokota et al., 1995; Yoshihara et al., 1996).

Our laboratory employed the technique of arterial impedance analysis for a complete assessment of the arterial hemodynamics in spontaneously hypertensive rats (SHR) and normotensive Wister Kyoto rats (WKY) (Chen and Hu, 1997; Hu et al., 1994a, 1997). The aortic pressure and flow waves were subjected to frequency domain analysis to obtain the impedance spectra. In addition to the steady components of hemodynamics such as aortic pressure, cardiac output, and total peripheral resistance, several pulsatile hemodynamics including characteristic impedance, arterial compliance, and pulse wave reflection can be derived from the analysis. We found that in SHR, the aortic pressure, total peripheral resistance, impedance and pulse wave reflection were markedly increased compared with the WKY; yet the degree of VH was more closely correlated with the pulsatile (characteristic impedance and pulse wave reflection) than with the steady hemodynamics such as aortic pressure and total peripheral resistance (Hu et al., 1994a). In addition, acute block of NO formation affected mainly the steady hemodynamics with a slight effect on the pulsatile component (Chen and Hu, 1997; Hu et al., 1997).

In this study, we treated rats with various doses of L-NAME for a period of 8 weeks and calculated the LVW, LVW/BW, and morphological changes of cardiac myocytes as indices of the degree of VH. We also measured the contents for left ventricular (LV) collagen volume fraction (LVCVF), cGMP, and nitrite/nitrate to determine whether VH development was related to the NO-cGMP pathway. The purpose was to analyze the effects of chronic NOS blockade on the arterial hemodynamics and the possible role of hemodynamic parameters and chemical factors in the development of VH following chronic NO deprivation. To the best of our knowledge, the association of VH caused by chronic NO deprivation with the hemodynamic and biochemical factors has not been systemically evaluated.

## Materials and methods

### *Animals*

Rats of Sprague–Dawley (SD) strain were supplied by the National Animal Center and housed in an environmentally controlled room. The protocol of animal experiments was approved by the University Laboratory Animal Research Committee. Tail cuff pressure (TCP) was measured with a photoelectric volume oscillometer (Ueda, UR-5000 Tokyo, Japan).

### *Chronic L-NAME*

Rats aged 10-weeks old were given L-NAME 10, 20, or 40 mg/kg per day. The drug was dissolved in saline solution and administered orally via a gastric tube. Twelve rats received vehicle and served as the control group. Both L-NAME and vehicle were given for a period of 8 weeks, during which several L-NAME-treated rats died after exhibiting signs of stroke. The data in some rats were not used because of the surgical procedure to be described later. We added more animals until the number in each group reached twelve. The age of experimental and control rats was 18-weeks old at the time of entering the acute experiment.

### *Experimental preparation*

To prepare for the acute experiment, each rat was anesthetized with pentobarbital sodium (40 mg/kg) given intraperitoneally. A tracheotomy was performed to provide artificial ventilation with a tidal volume of 3–5 ml and a respiratory rate of 50–70 breaths/min. The femoral artery was cannulated for the recording of peripheral arterial pressure, and the femoral vein for the administration of drugs or fluid. Heart rate (HR) was monitored by a tachometer triggered by arterial pulses.

The aortic pressure flow waves were recorded for the arterial impedance analysis. Following anesthesia and artificial respiration, the chest was opened through the left third intercostal space. An electromagnetic flow probe (Carolina Medical Electronics, Model 100 series, internal circumference 7–9 mm) was placed around the ascending aorta to measure the aortic flow. A Millar catheter with one high-fidelity pressure sensor (Millar Instruments, Model SPR-407, Size 2F) was used to measure aortic pressure. In order to minimize baseline drift, the catheter was soaked in saline at room temperature for at least one hour before insertion. The Millar catheter was inserted via the isolated right carotid artery into the ascending aorta until the catheter tip reached a position just distal to the flow probe. An electrocardiogram (ECG) of lead II was recorded with an ECG/Biotech amplifier (Gould Instruments, Cleveland, Ohio, USA).

The aortic pressure, flow waves, and ECG were continuously monitored with a polygraph recorder (Power Lab, AD Instruments, Mountain View, Calif., USA) and were also recorded on a tape recorder (TEAC, Model MR-30) at a