Nitric Oxide–Dependent Vasodilation in Young Spontaneously Hypertensive Rats

Alberto Radaelli, Luca Mircoli, Ileana Mori, Giuseppe Mancia, Alberto U. Ferrari

Abstract—Conflicting evidence exists on the possible impairment of tonic nitric oxide (NO)–mediated vasodilation as a causative factor in the genesis of human as well as experimental hypertension. We evaluated the tonic NO-dependent vasodilation from the pressor response to NO synthesis inhibition by N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) in 9 conscious, chronically instrumented spontaneously hypertensive rats (SHR) at 12 weeks of age, ie, during the early established hypertensive stage. Nine age-matched Wistar-Kyoto rats (WKY) were used as controls. The pressor responses to L-NMMA (100 \text{ mg} \cdot \text{kg}^{-1} \text{ IV bolus} plus 1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \text{ infusion for 60 minutes}) as well as to non–NO-dependent pressor stimuli, namely, vasopressin (2, 4, and 8 \text{ ng} \cdot \text{kg}^{-1}) and phenylephrine (0.5, 1, and 2 \text{ mg} \cdot \text{kg}^{-1}) given as IV boluses, were assessed both under control conditions and during suppression of autonomic reflexes by hexamethonium (30 \text{ mg} \cdot \text{kg}^{-1} \text{ IV bolus}+1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \text{ infusion}). Rather than being reduced, the pressor responses to L-NMMA were 39% and 71% larger in the control and areflexic conditions, respectively, than those observed in WKY (both \( P<0.01 \)). A similar pattern was observed for the pressor responses to vasopressin (+37% and +68% in the control and areflexic conditions, respectively; both \( P<0.01 \)) and phenylephrine, (+20% and +52%; both \( P<0.05 \)). Additional groups of 6-week-old prehypertensive SHR (\( n=11 \)) and age-matched WKY (\( n=11 \)) were subjected to an identical protocol: in these animals, the pressor responses to L-NMMA were similar in each strain, as were the pressor responses to vasopressin and phenylephrine in both control and areflexic conditions. In conclusion, our observations indicate that during the developmental phase of hypertension in the SHR model, namely, during the prehypertensive as well as the early established hypertensive stage, NO-dependent vasodilation is preserved (if not enhanced) so that a putative impairment of this function provides no significant pathogenic contribution to the onset of hypertension in this experimental model. (Hypertension. 1998;32:735-739.)

Key Words: nitric oxide ■ rats, inbred SHR ■ vascular reactivity ■ L-NAME ■ hexamethonium

Growing attention is being given to endothelial function in hypertension and to the possibility that an inadequate nitric oxide (NO)–mediated vasodilation may be of pathogenic importance in the onset and maintenance of the hemodynamic hallmark of the disease, ie, the elevation in peripheral vascular resistance. Data on this issue are controversial, however, because altered vasomotor responses to both activation and inhibition of NO release have been reported in the human forearm or coronary bed,\textsuperscript{1-5} and data conveying the same message have been obtained in small isolated vessels from either essentially hypertensive patients or spontaneously hypertensive rats (SHR).\textsuperscript{6-9} In contrast, no endothelial dysfunction was detected in hypertensive subjects by other investigators,\textsuperscript{10-13} and biochemical evaluations of the NO system (NO synthases, plasma and urine nitrates and nitrites, plasma and urine cGMP) have failed to show any impairment in either experimental or human hypertension.\textsuperscript{14-16}

The discrepant conclusions reached by the above-mentioned reports may depend at least partly on limitations that inherently characterize human studies on endothelial function in hypertension, such as the use of an artificial stimulus (intra-arterial acetylcholine or methacholine), restriction of the hemodynamic assessment to regional vascular beds, and extrapolation from in vitro experiments.

A number of limitations, however, have also characterized studies on endothelial function in hypertensive animal models because of the use of anesthetized preparations\textsuperscript{17-20} and the failure to take into account other potential confounders, such as the reflex modulation of the pressor response to administration of L-arginine analogues\textsuperscript{17,21} or the nonspecific vascular hyperreactivity\textsuperscript{17,19-21} that is typical of the hypertensive condition.\textsuperscript{22,23} In the present study, we measured the systemic pressor effect of acute NO synthase inhibition by N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) under experimental conditions that avoided the above limitations, ie, by use of conscious SHR either with an intact autonomic function or made areflexic by ganglionic blockade and concurrently subjected to evaluation of vascular reactivity. The data were collected...
in prehypertensive and hypertensive SHR so as to have an insight into the dynamics of endothelial function during the development of hypertension.

**Methods**

**Animal Preparation and Surgery**

The study was conducted on 23 Wistar-Kyoto rats (WKY) and 17 SHR (Charles River Italia SpA, Calco, Italy) at 12 weeks of age. Additional groups of 11 WKY and 11 SHR were studied at the age of 6 weeks, ie, during a stage regarded to be largely prehypertensive.

In each rat, polyethylene catheters were implanted unilaterally in the femoral artery for arterial blood pressure recording and bilaterally in the femoral veins for injection of different drugs. The catheters were tunneled subcutaneously, exteriorized at the dorsal neck region, and kept patent by flushing with low-concentration heparin solution (0.01% vol/vol). After surgery, at least 24 hours were allowed for the animal to recover and acclimate to the experimental environment, which consisted of a wide cage in which the rat could walk, explore, eat, and drink ad libitum.

All procedures were in accordance with guidelines of the Italian government concerning the protection of animals used for scientific purposes.

**Experimental Protocol**

All experimental sessions were performed during the day. The arterial catheter was connected to a P23Dc pressure transducer (Gould-Statham) that had a flat frequency response up to 30 Hz. The blood pressure signal was continuously displayed on a chart recorder (7D polygraph, Grass Instruments). Heart rate was derived from the pulsatile pressor signal via tachographic beat-to-beat conversion.

After a 30-minute period of equilibration, the pressor response to administration of the NO synthesis inhibitor L-NMMA was assessed. The dose of L-NMMA was a 100 mg kg⁻¹ IV bolus followed by a 1.5 mg kg⁻¹ min⁻¹ infusion that was maintained for 25 to 30 minutes. Total volume of injected fluid (bolus+infusion) was <1.5 mL.

In both WKY and SHR, the pressor response to L-NMMA was also evaluated 24 hours later, after acute administration of the ganglion blocker hexamethonium as an intravenous bolus dose of 30 mg kg⁻¹ followed by a 1.5 mg kg⁻¹ min⁻¹ infusion. This made the animal areflexic and avoided possible confounding effects of the baroreflex-mediated cardiovascular adjustments elicited by the blood pressure elevation that follows administration of L-NMMA. Suppression of reflex influences by this treatment was demonstrated in preliminary experiments (n=5) demonstrating that a bolus of phenylephrine (4 mg kg⁻¹) administered before hexamethonium produced a rise in mean arterial pressure of 47.0±2.4 mm Hg and a reduction in heart rate of 51.0±8.5 bpm, whereas after hexamethonium the blood pressure increase was 53.0±3.7 mm Hg and the reduction in heart rate was 5.0±2.0 bpm. To limit the hexamethonium-induced fall in blood pressure, all animals were given a plasma expander (Eufusin, 4.5 mL kg⁻¹ h⁻¹). The L-NMMA was administered 20 minutes after the hexamethonium infusion was started, at the same dose used on the previous day.

In both 12- and 6-week-old SHR and WKY, the pressor responses to graded doses of vasopressin (2.1, 4.2, and 8.3 ng kg⁻¹) and phenylephrine (0.5, 1, and 2 µg kg⁻¹) were also assessed to determine whether nonspecific differences in vascular reactivity to pressor agents existed between the 2 strains.

Because administration of hexamethonium lowered blood pressure to a greater extent in 12-week-old WKY than in SHR (thus further widening the already marked difference in baseline pressure between the 2 groups), 6 additional hexamethonium-infused 12-week-old WKY were given L-NMMA during an infusion of arginine-vasopressin (0.415 µg kg⁻¹ min⁻¹) administered before hexamethonium to restore a concordant vasopressin effect (hexamethonium+AVP). Values below each bar (mean±SEM) refer to baseline MAP; note the similar baseline MAP in hexamethonium+AVP–treated WKY and in hexamethonium-treated SHR.

**Figure 1.** Original recordings of the pressor responses to L-NMMA (arrow marks time of injection) in 12-week-old WKY and SHR studied in the control (intact, upper panels) and areflexic condition (hexamethonium, lower panels). ABP indicates pulsatile arterial pressure; HR, heart rate.

**Figure 2.** Mean arterial pressure responses (ΔMAP) to L-NMMA in the control (intact, top) and areflexic (hexamethonium, middle) conditions in the whole groups of 12-week-old WKY (open bars) and SHR (hatched bars). Bottom, Areflexic 12-week-old SHR are compared with areflexic WKY with their blood pressure restored by a concurrent vasopressin infusion (hexamethonium+AVP). Values below each bar (mean±SEM) refer to baseline MAP; note the similar baseline MAP in hexamethonium+AVP–treated WKY and in hexamethonium-treated SHR.
ng · kg⁻¹ · min⁻¹), which restored blood pressure to values similar to those of ganglion-blocked 12-week-old SHR.

Data Analysis

According to the purpose of the study, interstrain comparisons of age-matched rats were performed, whereas intrastrain differences of younger versus older rats were not considered. Baseline blood pressure and pulse rate, defined as the average values during the 5 minutes preceding L-NMMA, vasopressin, or phenylephrine injection, were compared with the average values observed at between 5 and 10 minutes after injection (L-NMMA) or at the peak of the transient pressor response (vasopressin and phenylephrine). The statistical significance of the between-strain and between-drug differences in response to the various experimental interventions was assessed by the paired or unpaired Student’s t test, with application of the Bonferroni correction whenever multiple comparisons were made. The level of statistical significance was set at P<0.05.

Results

Established Hypertensive Rats

Compared with that of intact rats, baseline mean arterial pressure was, as expected, significantly higher in SHR than in WKY (136±4 versus 99±4 mm Hg, respectively; P<0.01), whereas heart rate was similar in the 2 groups (330±14 versus 336±13 bpm, respectively; P=NS). The difference in mean arterial pressure between the SHR and WKY was still evident in the ganglion-blocked condition (109±4 versus 61±5 mm Hg, respectively; P<0.01).

As shown in the examples of Figure 1 and in the average data of Figure 2 (top and middle), inhibition of NO synthesis by L-NMMA produced significant and marked blood pressure increases in both WKY and SHR, both without and with ganglionic blockade. Rather than being attenuated, the pressor response to L-NMMA was greater in SHR than in WKY, the difference being significant and marked both in the control condition and during suppression of autonomic reflexes by hexamethonium. The enhanced pressor response to L-NMMA in SHR was maintained when the comparison was made between ganglion-blocked SHR and ganglion-blocked WKY in which blood pressure was restored by a vasopressin infusion (Figure 2, bottom).

As shown in the examples of Figure 1, the bradycardic response accompanying the L-NMMA–induced blood pressure rise was well evident in intact WKY and SHR, although somewhat less pronounced in the latter (−88.0±8.0 and −76.6±11.5 bpm, respectively) but was virtually abolished in animals subjected to concurrent hexamethonium infusion (−8.0±3.4 and −7.5±3.6 bpm).

The pressor responses to the graded bolus doses of phenylephrine and vasopressin showed a pattern similar to those to L-NMMA, namely, a greater response in SHR compared with WKY in either intact or hexamethonium-treated rats. This is exemplified in Figure 3 (phenylephrine injections in 12-week-old WKY and SHR) and illustrated in detail in Figure 4. Heart rate responses also largely paralleled those observed in the L-NMMA experiments (data not shown).

Prehypertensive Rats

At variance with the older animals, the 6-week-old SHR had only moderately (although already significantly) elevated baseline mean arterial pressure compared with age-matched WKY, the respective group values being 107±4.2 versus 90±2.5 mm Hg (P<0.05). The difference was again main-
tained during ganglionic blockade (75±5 versus 56±3 mm Hg, respectively; *P*, 0.05).

No differences in the pressor responses to L-NMMA could be detected in the 6-week-old SHR compared with age-matched WKY either without or with ganglionic blockade (Figures 5 and 6). These groups of rats also displayed pressor responses similar to those to the graded bolus doses of phenylephrine and vasopressin (Figure 4).

**Discussion**

Our data show that in 11- to 12-week-old conscious SHR, the pressor response to L-NMMA is markedly greater than that seen in age-matched WKY, both in the intact condition and after the animals were made areflexic by ganglionic blockade. They further show, however, that the enhanced pressor response to L-NMMA is paralleled by a quantitatively similar enhancement of the pressor response to both phenylephrine and vasopressin, the SHR/WKY difference being evident again in both intact (39%, 20%, and 37% for L-NMMA, phenylephrine, and vasopressin, respectively) and ganglion-blocked rats (71%, 52%, and 68%, respectively). The data also show that in younger intact or ganglion-blocked SHR, in which the blood pressure elevation was no more than borderline, the pressor response to L-NMMA was similar to that of age-matched WKY, with an unaltered pressor response also to the other 2 vasoconstrictor agents tested.

Taken together, these findings allow us to conclude that the tonic contribution of NO to blood pressure modulation is not impaired in young SHR, which stands against any substantial involvement of a dysfunction of the NO system in the pathogenesis of the blood pressure elevation in this strain. The findings also allow us to suggest that in this hypertensive model, an impairment of NO-dependent vasomotor control is unlikely to occur even at a later stage when blood pressure is markedly elevated. Indeed, under this circumstance, blood pressure response to L-NMMA is exaggerated rather than depressed. This, however, should not be interpreted as evidence of a hyperfunctioning NO system; rather, it likely reflects nonspecific vascular hyperreactivity to many vasoconstrictor stimuli that is typical of the hypertensive condition.

A few further issues raised by our experiments need to be discussed. First, as mentioned above, our evidence that there is no impairment of tonic NO release leading to chronic blood pressure elevation applies to young SHR. We obviously cannot exclude that a deficient production of NO occurs in hypertensive humans, in other experimental models of hypertension, and in later stages of the disease in SHR themselves. This may be because the long-standing mechanical trauma may eventually damage endothelial cells and because this damage is easily caused by conditions (eg, hypercholesterolemia,25 diabetes,26 atherosclerosis) frequently associated with long-standing blood pressure elevations.27 Second, our findings cannot exclude that other components of endothelial function influencing vasomotor tone, such as endothelin,28 the so-called endothelium-derived hyperpolarizing factor,29 and prostaglandins,30,31 may be affected in the early hypertensive stage even in the SHR model and may be of greater pathogenic relevance than NO release. Finally, in our experiments we inferred NO-dependent vasodilation from the measurement of intra-arterial blood pressure without attempting to directly assess peripheral resistance by concomitantly measuring cardiac output. It was shown in previous studies, however, that the cardiac output change associated with NO synthesis inhibition (1) consists of a decrease that is observed in both the intact and the denervated heart32 and (2) is largely similar in SHR and WKY.32,33 Consequently, there should be no question that the blood pressure changes we measured in response to L-NMMA did reflect an increase in systemic vascular tone (whose magnitude may, if anything, have been somewhat underestimated) and that, to the aim of the comparison between SHR and WKY, the observed changes in blood pressure were reliable proportional indicators of the concomitant changes in systemic vascular resistance.
In conclusion, our observations indicate that during the developmental phase of hypertension in the SHR model, namely, during the prehypertensive stage as well as the early established hypertensive stage, NO-dependent vasodilation is unimpaired. Therefore, it is evident that a putative dysfunction of this system provides no significant pathogenic contribution to the onset of hypertension in this experimental model.

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