Ambulatory Cardiovascular Activities in L-NAME-Treated Mice

Jong Y. Lee* and Silvia H. Azar

Department of Medicine, University of Minnesota School of Medicine, Minneapolis, Minnesota, USA

Abstract: *Objective*: High blood pressure (BP) is a dominant risk factor in cardiovascular diseases. An experimental model of nitric oxide synthase (NOS) inhibitor induced hypertension was developed to study some etiologic mechanisms in cardiovascular parameters.

Methods: Cardiovascular rhythm characteristics were documented in mice following the N-omega-nitro-L-arginine-methyl-ester (L-NAME)-treatment (Rx). Radio-telemetered BP, heart rate (HR), and locomotor activity (LA) were measured every 4 min for 5 days before and for 14 days after Rx. Data was converted into an hourly average and analyzed by the linear least square rhythmometry.

Results: L-NAME-Rx increased systolic BP (SBP) significantly without significant changes in diastolic BP and markedly reduced HR: SBP (mm Hg) 143.4 ± 0.6 *versus* 148.9 ± 0.4, P <0.0001; HR (beat/min): 552.13 ± 2.7 *vs*. 481 ± 1.8, P <0.0001, with markedly depleted amplitude. SBP variations were mainly during the night time, while HR variations were almost every time-point comparison throughout the 24-h span. Although the overall LA was not significantly changed with L-NAME-Rx, time-point depleted LA was noted, especially when the light was off at 18:00 hour through midnight (P <0.0001), while an opposite result was observed at noon with significantly increased LA in this nocturnal animal (P <0.005), with markedly decreased amplitude (P <0.01). Interestingly, we observe reduced HR with L-NAME-Rx contradicted to other reports.

Conclusion: The results suggest that the NOS blockade may impair cardiovascular autonomic adaptations and arterial baroreflex integration, resulting in an increased vascular tone during the systole, but not an end diastole in the relaxed cardiac autonomic tonus.

Keywords: Ambulatory blood pressure, L-NAME, nitric oxide synthase, heart rate, locomotor activity, circadian rhythmicity.

INTRODUCTION

Rats and mice are commonly used in experimental animal models to assess and evaluate human hypertension and cardiovascular activities. Various rats were used in our ambulatory blood pressure monitoring studies and also in some other groups [1-4]. The mouse is a popular subject of study, helping understand human cardiovascular diseases even though the mechanisms of regulating cardiovascular function in this animal have not yet been fully understood.

The nitric oxide (NO) pathway plays a central role in maintaining physiological organ functions. There is a positive correlation between NO bioavailability and maximal oxygen uptake, as seen in the significant increase in NO production in elite soccer players [5]. Reduced NO causes endothelial and right heart dysfunctions and pulmonary vascular pruning [6]. The disruption of NO, resulting in a reduction of NO bioavailability, impairs smooth muscle relaxation, and blood pressure (BP) and hemorheological values are normalized when treated with angiotensin (Ang) AT1 receptor antagonist valsartan [7]. Superoxide dismutase 1-knockout or transgenic mice limit renal microvascular remodeling and attenuates arteriole and BP responses to Ang II via modulation of NO bioavailability [8].

The regulation of NO functions is supported by a response to oxygen and redox agents that affects the molecular and functional structures of the blood cell proteins. The erythrocyte membrane-bound NO mediates hypoxic vasodilation by transporting and releasing NO bioactivity to areas of tissue hypoxia, although the mechanisms of cardiovascular function involved in NO synthase (NOS) are not well understood. However, the final metabolic fate of heme-bound NO (methemoglobin) is conversion into nitrate, eliminating the availability of NO in blood circulation, while NO also reacts with hemoglobin (Hb) to form stable metabolites to transport and release NO to distant areas [9,10].

The underlying mechanism in the role of oxidative stress and epigenetic changes may cause vascular dysfunctions, which impair endothelium-mediated vasodilation and cause cardiovascular-related diseases [11]. Besides genetics, the perinatal environment, especially in a perinatal low protein (LP) diet, contributes to elevated BP and renal dysfunctions in later life [1,12]. Birth weight and later growth were markedly lower in LP offspring with increased mean arterial pressure, as well as the decreased NO-mediated vascular response as compared to the control group. Endothelial endothelin B receptors-mediated pathway of vascular relaxation involving release of NO, whch may protect against the excessive vasoconstriction and increased BP caused by a high salt diet [13].

*Address correspondence to this author at the Department of Medicine, University of Minnesota School of Medicine, Minnesota, P.O. Box 14945, Minneapolis, MN 55414, USA; Tel: (612) 408-3125; Fax: (612) 379-2467; E-mail: leexx154@umn.edu

1876-5262/11

Although many studies have been carried out in N-omega-nitro-L-arginine-methyl-ester (L-NAME) induced hypertension, ambulatory cardiovascular activities with L-NAME treatment have not been extensively studied. The BP and heart rate (HR) in both intact and knockout endothelial NOS (eNOS) strains were markedly affected by brief locomotor activity (LA) cycles with a short term L-NAME measurement [14]. L-NAME-treated mice became hypertensive with enhanced baroreflex and chemoreflex, while showing no differences in heart rates under urethane anesthesia [15]. Thus, we investigated the effect of NOS inhibition on circadian rhythm characteristics of BP, HR, respiratory rate (RR) and LA in the intact eNOS mice for a lengthy telemetry monitoring before and after the L-NAME treatment to see the effects of daily basis changes for the entire treatment period. Before starting L-NAME experiments, post-surgery daily ambulatory monitoring during the recovery period was also performed until synchronized circadian rhythm characteristics were established. Telemetry monitoring studies using mice were not fully developed due to the limited weight of mice, and we helped the Dataquest telemetry system (Data Sciences International, St. Paul, MN) to develop the telemetered monitoring in mice before this current monitoring study.

MATERIAL AND METHODOLOY

1. Animals

About 10-11 week-old male mice with the intact eNOS (wild-type C57BL/6, purchased from Harlan, Indianapolis, IN) were housed at the University animal facilities on a 12h light-dark cycle (light: 06:00 to 18:00) and had free access to food and water for a week before telemetry monitoring procedures, and maintained under the same condition following surgery. The study was approved by the institutional animal care and use committee, and all of the study protocols followed by the National Institutes of Health and the University of Minnesota Animal Use guidelines.

2. Procedures

2.1. Telemetry Transmitter Implants

Radiotelemetry transmitter installation surgery was carried out after the mice were stabilized following arrival. All surgical procedures and techniques were established and used extensively in our laboratories [1-3,16-18]. Mice were placed on a warm pad during the surgery and sterile methods were used under methohexical anesthesia for the aortic catheter placement and the abdominal wall body affixing of the transmitters [2]. An abdominal incision was made through the midline to access the aorta caudally to the left renal artery. The catheter's entry point was secured with Vetbond tissue adhesive No. 1469 (3M Animal Care Products. St. Paul, MN, USA) and a 0.2 x 0.3 cm cellulose fiber patch, and the transmitter was affixed to the inner peritoneal wall at the midline incision with non-absorbable polypropylene sutures. The system's accuracy was also tested by an arterial cannula, placed less than 1 cm from the transmitter cannula location, connected to a mercury sphygmomanometer measured simultaneously with the telemetered BP under anesthesia. Following surgery, the mice were placed in warm boxes and received about 0.5 ml acetaminophen (0.96 mg/I ml water), followed by about 1 ml of 0.45% NaCl/5% glucose solution. The mice were returned to their home cage when they were fully awake and mobile, and were offered freely accessible food with acetaminophen in 5% glucose solution for the first 24 hours post-surgery, followed by tap water. The ambulatory monitoring system in mice was not well established until this study. Since the telemetry methodology development was part of this study, their surgery recovery status was monitored daily to document the completely synchronized recovery from surgery. Thus, we carried out post-surgery recovery monitoring in BP and HR for 4 weeks to establish the reliable data collecting system before the actual L-NAME study began (Fig. **1**).

2.2. L-NAME Treatment and Data Collection

Mice were treated with NOS inhibitor, L-NAME (60 mg/dl in drinking water, Sigma Chemical Co, MO) [7,19,20] for 10 days to examine the effects on ambulatory cardiovascular activities. Radio-telemetered BP, HR, RR and LA levels were measured every 4 min for 5 days before and for 14 days after L-NAME-treatments, and circadian (MESOR: rhythm adjusted 24-h mean, midline estimating statistic of rhythm) data was collected by the Data Quest Program (Data Science International, St. Paul, MN) [2].

2.3. Statistics

Telemetered data was converted into hourly averages, and analyzed by a paired *t-test* method and by the linear least square rhythmometry to obtain MESOR and other rhythm characteristics [21]. Data is expressed as mean ± SEM. A p value of less than 0.05 was considered significant.

RESULTS

The circadian variables were monitored closely following the telemetry surgery to ensure the synchronized data collection. As part of the cardiovascular monitoring methodology development, the MESOR of variables was monitored following post-surgery recovery for 4 weeks before the actual L-NAME study. At post-surgery day-2, the data was very unstable with no established rhythm and amplitude (a measure of one-half the extent of predictable change within a day, or half of the total predictable change in rhythms defined by a rhythmic function fitted to data) was homogeneously low (Fig. **1**). Acrophase (a measure of the timing of overall high values recurring each cycle in relation to a reference time, local midnight) was in all over the directions. Acrophase occurred during daytime hours for both systolic BP (SBP) and HR at day-2 in this nocturnal animal (Fig. **1**). However, the acrophase of all mice showed at nighttime hours by day-4 and mean peak hour was found to be around 22:40 for SBP and 20.56 for HR, though there were still some individual variations (Table **1**). This value was close to the acrophase value achieved at week-4 for SBP (23:12) and HR (21:29) (Table **1**). As shown in Table **1**, **5** individual mice on day-4 post-surgery HR showed shallow and unstable amplitudes, with various rhythm characteristics noted during post-surgery day-1 through day-4. Meanwhile, fair diurnal rhythms were established on post-surgery day-5 for both SBP and HR, although with various MESORs and shallow amplitudes, especially in HR. For the day-7 and week-4 data comparison, some unstable rhythms were still

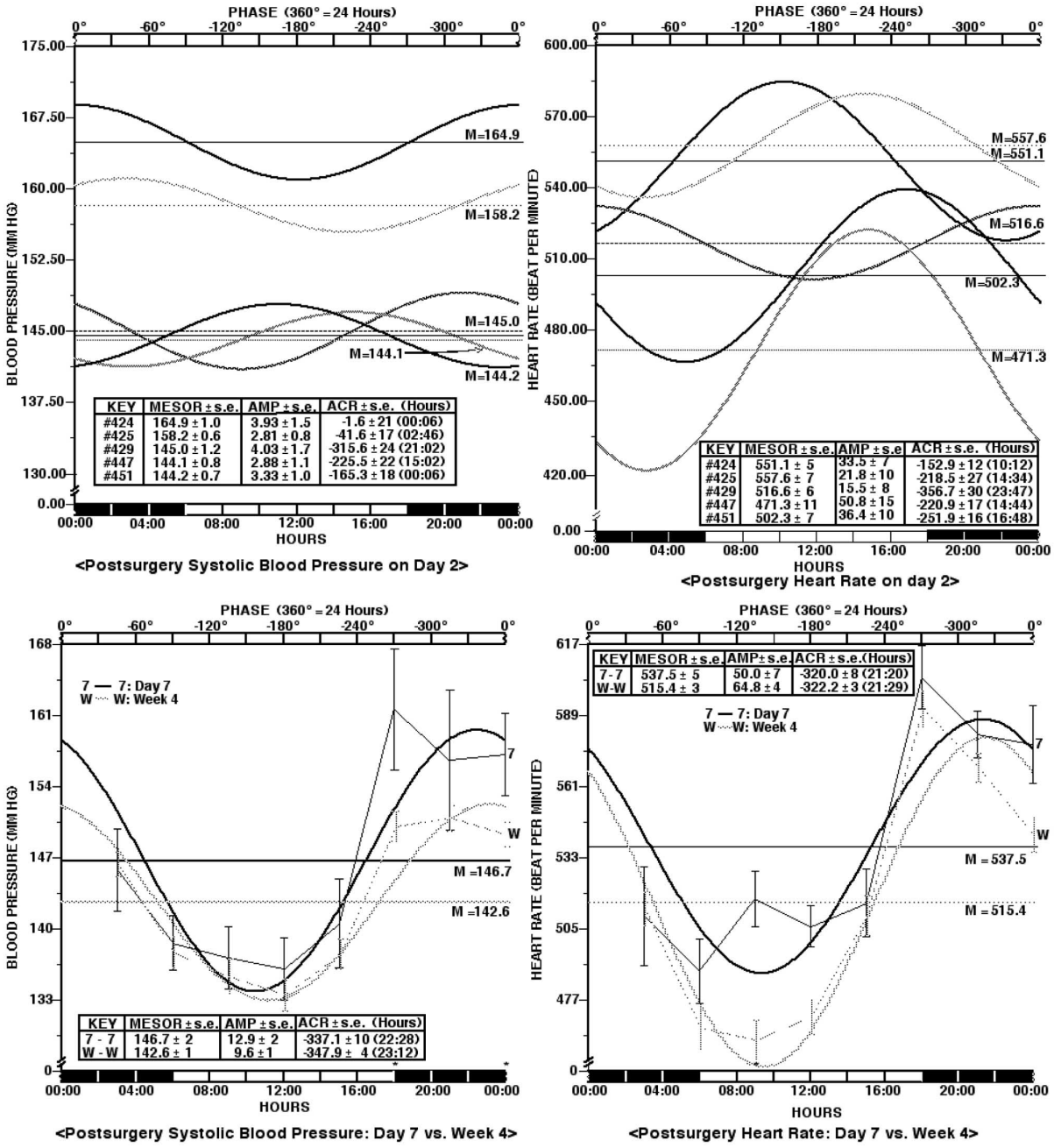


Fig. (1). Postsurgery recovery monitoring in blood pressure and heart rate in 5 mice. As part of the cardiovascular monitoring methodology development, post-surgery blood pressure and heart rate were monitored for 4 weeks before the actual L-NAME study. Every 4-min telemetered data were collected using the Data Quest Program (Data Science International, St. Paul, MN) [2] and converted into hourly averages, and analyzed by the linear least square rhythmometry [21]. Upper panel: mice at post-surgery day-2 show very unstable data with no established rhythm; Lower panel: a comparison in the post-surgery day-7 and week-4 groups. Some unstable rhythms are still noted at day-7 as compared with data from week-4. M = MESOR: a rhythm-adjusted 24-hour mean (midline estimating statistic of rhythm). Amplitude: a measure of one-half the extent of predictable change within a day, or half of total predictable change in rhythm defined by rhythmic functions fitted to data. Acrophase: a measure of the timing of overall high values recurring each cycle in relation to a reference time (local midnight). #424, #425, #429, $447 and #451: assigned rat number: rat numbers. *P <0.05.

noted at day-7, with a limited synchronization of BP and HR, especially in amplitude comparisons (Fig. **1** and Table **1**). Day-9 circadian characteristics showed no greater statistical differences when compared to week-4 data. Thus, MESOR of SBP and each 3-hour time point of circadian values were similar to those of week-4 values by day-9 post surgery (Table **1**). Amplitude was homogeneously low at day-2 post surgery for SBP with wildly swinging heart rates (Fig. **1**), but stabilized by day-9 (Table **1**). Thus, a circadian rhythm was resynchronized by this time following surgery.

Cardiovascular rhythms are mostly stabilized after day-9 post-surgery, as the current study documented and verified (Table **1**). Thus, this study establishes a reliable post-surgery

ambulatory cardiovascular monitoring methodology before entering the L-NAME study (Fig. **1**, Table **1**).

L-NAME treatment increases SBP and decreases HR while reducing the amplitude of the locomotor activity in the current study. In Table **2** and Fig. (**2**), the L-NAME treatment increased systolic BP (SBP) and delayed acrophase (peak time) significantly: SBP (mm Hg): 143.4 ± 0.6 *versus* 148.9 ± 0.4, F-value = 63.167, P < 0.0001; acrophase (in hour) 23:13 *versus* 00:37, F-value = 36.143, P < 0.0001. Although the diastolic BP (DBP) level was not significantly changed (102.2 *versus* 101.5 mm Hg, P = not significant), acrophase was significantly delayed by L-NAME treatment: 21:18 *versus* 23:31 (hour), F = 11.223, P < 0.001. No apparent change in amplitude was noted in either SBP or DBP.

Table 1. Circadian Rhythm Characteristics of Systolic Blood Pressure and Heart Rate after Surgery

Time		MESOR		Amplitude		Acrophase	
		SBP	HR	SBP	HR	SBP (Hours)	HR (Hours)
Day 4	#424	156±2	542±06	8.4+3	08±08	-314±19 (20:56)	-274±53 (18:16)
	#425	154±8	531±08	6.4±1	17±11	-18±10 (01:12)	-346±37 (23:04)
	#429	143±2	515±08	11.0±3	30±12	-313±16 (20:52)	-311±22 (20:44)
	#447	147±9	484±09	4.7±1	40±13	-311±15 (20:44)	-310±18 (20:40)
	#451	150±6	563±07	7.0±8	44±10	-349±07 (23:16)	-331±13 (22:04)
Day 5	n=5	151±1‡	534±05†	10.2±2	29±07‡	-340±10 (22:40)	-322±14 (21:28)
Day 6	n=5	148±2†	531±06	11.5±2	48±09	-337±10 (22:28)	-316±10 (21:04)
Day 7	n=5	147±2*	537±05‡	12.9±2	50±07	-337±10 (22:28)	-320±03 (21:20)
Day 8	n=5	147±2*	522±06	15.9±2*	63±08	-337±08 (22:28)	-325±08 (21:40)
Day 9	n=5	142±1	522±06	9.2±1	55±07	-338±07 (22:32)	-324±07 (21:36)
Week 4	n=5	143±1	515±03	9.6±1	65±04	-348±04 (23:12)	-322±03 (21:29)

#424, #425, #429, $447 and #451: assigned rat number; SBP: systolic blood pressure; HR: heart rate.
*P < 0.05; **P < 0.01; †P < 0.001; ‡P < 0.0001, as compared to week-4 values.
MESOR: a rhythm-adjusted 24-h mean, midline estimating statistic of rhythm.
Amplitude: a measure of one-half the extent of predictable change within a day, or half of total predictable change in rhythm defined by rhythmic functions fitted to data.
Acrophase: a measure of the timing of overall high values recurring each cycle in relation to a reference time (local midnight).

Table 2. Cardiovascular Rhythm Characteristics before and after L-NAME Treatment

Variables		MESOR	Double Amplitude	Acrophase (Hour)
SBP (mm Hg):	Control	143.4 ± 0.6	20.1 ± 0.9	-333.3 (23:13) ± 5
	Treated	148.9 ± 0.4	20.2 ± 0.5	-9.4 (00:37) ± 3
	F-value	63.167	0.002	36.143
	P-value	< 0.0001	NS	< 0.0001
DBP (mm Hg):	Control	102.2 ± 0.6	10.8 ± 0.8	-319.5 (21:18) ± 9
	Treated	101.5 ± 0.3	13.4 ± 0.4	-352.7 (23:31) ± 3
	F-value	1.226	2.179	11.223
	P-value	NS	NS	< 0.001
HR (beat/min):	Control	552.1 ± 2.7	132.0 ± 3.8	-312.7 (20:51) ± 3
	Treated	481.4 ± 1.8	112.4 ± 2.5	-332.6 (22:10) ± 3
	F-value	495.337	5.856	22.000
	P-value	< 0.0001	< 0.02	< 0.0001
RR (count/min):	Control	121.9 ± 0.6	15.7 ± 0.8	-327.9 (21:52) ± 6
	Treated	123.8 ± 0.3	16.9 ± 0.4	-1.5 (00:06) ± 3
	F-value	8.451	0.423	22.208
	P-value	< 0.005	NS	< 0.0001
LA (count/min):	Control	4.00 ± 0.2	4.34 ± 0.3	-352.7 (23:32) ± 7
	Treated	3.85 ± 0.1	2.86 ± 0.1	-358.5 (23:52) ± 4
	F-value	0.609	6.725	0.496
	P-value	NS	<0.01	NS

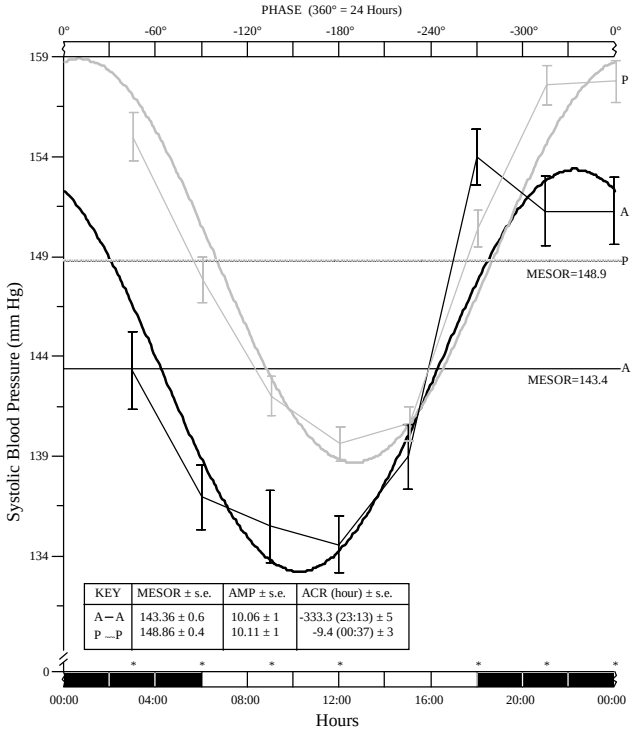
MESOR: a rhythm-adjusted 24-h mean.
Amplitude: a measure of one-half the extent of predictable change within a day, or half of total predictable change in rhythm defined by rhythmic functions fitted to data.
Acrophase: a measure of the timing of overall high values recurring each cycle in relation to a reference time (local midnight).
NS: statistically not significant; L-NAME: N-omega-nitro-L-arginine methyl ester; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate; LA: locomotor activity. Five mice were in each group throughout study span.

L-NAME treatment significantly reduced HR and delayed its peak time: 552.13 ± 2.7 *versus* 481 ± 1.8 (beat/min), F = 495.337, P < 0.001; acrophase (hour): 20:51 *versus* 22:10, F = 22.00, P < 0.0001. Significantly reduced double amplitude in HR was also noted: 132.0 *versus* 112.4 (beat/min), F = 5.856, P < 0.02. In RR comparisons, a slight but significantly increased RR and delayed phase were noted with L-NAME-treatment: 121.9 ± 0.6 ± 0.6 *versus* 123.8 ± 0.3 (count/min), F = 8.451, P < 0.005; Acrophase (hour): 21:52 *versus* 00:06, F = 22.208; P < 0.0001, while no apparent amplitude change was noted. No apparent LA change in MESOR and acrophase was noted following the L-NAME treatment. However, significantly reduced double amplitude was observed in L-NAME treatment: 4.34 ± 0.3 *versus* 2.86 ± 0.1 (count/min), F = 6.725; P < 0.01 (Table **2**, Fig. **2**).

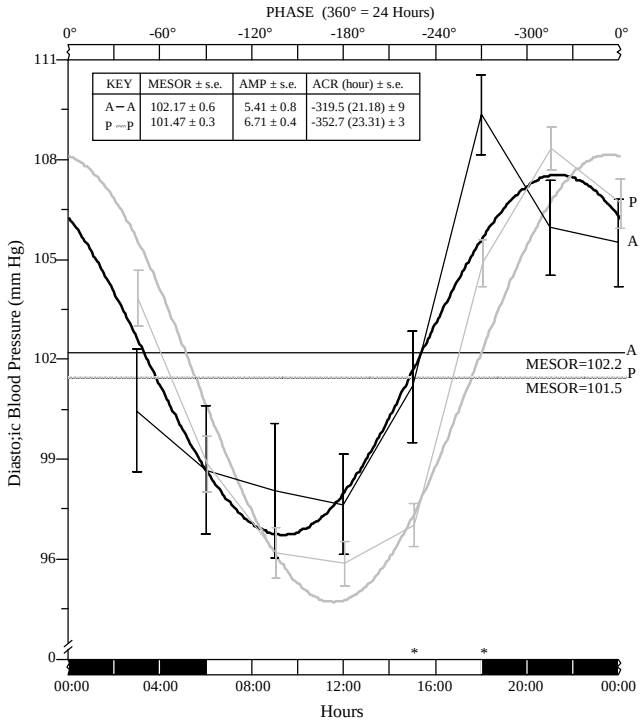
Table 3. Circadian Systolic Blood Pressure and Heart Rate Before and After L-NAME Treatment

RxT (n=5)	MESOR		Amplitude		Acrophase	
	SBP	HR	SBP	HR	SBP (Hours)	HR (Hours)
Basal *vs*.	143 ± 1	552 ± 03	10.1 ± 1	66 ± 04	-333 ± 05 (22:12)	-313 ± 03 (20:52)
Day 1	143 ± 1	514 ± 07¶	9.3 ± 2	94 ± 09‡	-334 ± 12 (22:16)	-314 ± 06 (20:56)
Day 2	149 ± 1¶	587 ± 06¶	7.8 ± 2	14 ± 09¶	- 5 ± 13 (00:20)‡	-60 ± 35 (04:00)¶
Day 3	139 ± 2†	498 ± 06¶	8.3 ± 2	94 ± 09‡	-331 ± 15 (22:04)	-303 ± 05 (20:12)
Day 4	152 ± 2¶	462 ± 07¶	12.4 ± 2	51 ± 10§	-12 ± 08 (00:48)‡	-320 ± 11 (21:20)
Day 5	152 ± 1¶	452 ± 06¶	10.9 ± 2	56 ± 08‡	-15 ± 09 (01:00)‡	-332 ± 08 (22:08)
Day 6	149 ± 1¶	459 ± 06¶	12.2 ± 2	58 ± 08*	-360 ± 09 (24:00)	-323 ± 08 (21:32)
Day 7	148 ± 2†	451 ± 05¶	12.3 ± 2	61 ± 08	- 8 ± 11 (00:32)‡	-328 ± 07 (21:52)
Day 8	149 ± 1¶	451 ± 06¶	10.1 ± 2	63 ± 09	-15 ± 12 (01:00)‡	-329 ± 08 (21:56)
Day 9	151 ± 1¶	453 ± 06¶	9.0 ± 2	59 ± 08	-26 ± 12 (01:44)‡	-322 ± 08 (21:28)
Day 10	149 ± 1¶	454 ± 11¶	9.4 ± 2	59 ± 09	-18 ± 12 (01:12)‡	-339 ± 09 (22:36)†
Day 11	150 ± 1¶	454 ± 06¶	11.4 ± 2	59 ± 09	- 6 ± 09 (00:24)‡	-339 ± 09 (22:36) †
Day 12	151 ± 2¶	475 ± 07¶	9.7 ± 2	60 ± 09	-13 ± 11 (00:52)‡	-345 ± 09 (23:00)‡
Day 13	151 ± 1¶	481 ± 06¶	11.0 ± 2	66 ± 08	-18 ± 10 (01:12)‡	-343 ± 07 (22:52)§
Day 14	148 ± 2†	496 ± 07¶	11.9 ± 3	60 ± 10	-346 ± 12 (23:04)	-333 ± 09 (22:12)*

Rx T: L-NAME treatment time; n = rat number; SBP = systolic blood pressure (mm Hg); HR = heart rate (beat/min);
*P < 0.05; †P < 0.01; ‡P < 0.005; §P < 0.001; ¶P < 0.0001



<Figure 2a. Systolic Blood Pressure Comparison >



<Figure 2b. Diastolic Blood Pressure Comparison >

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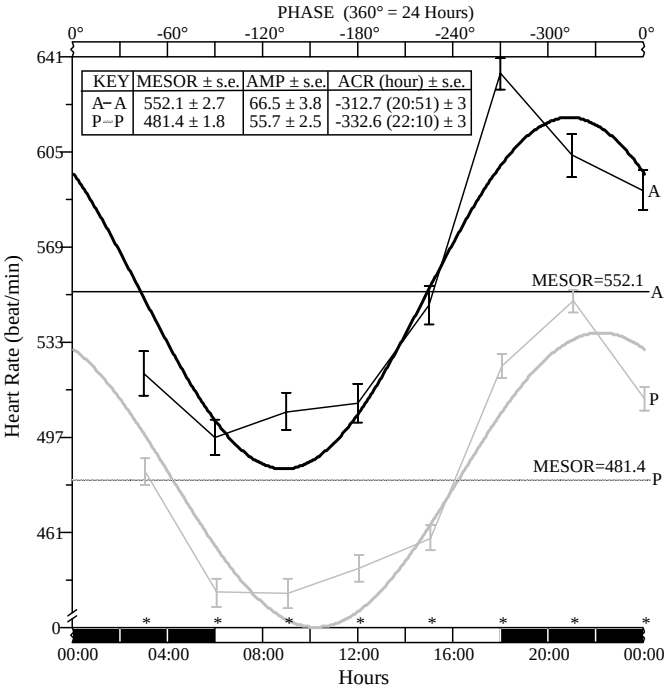
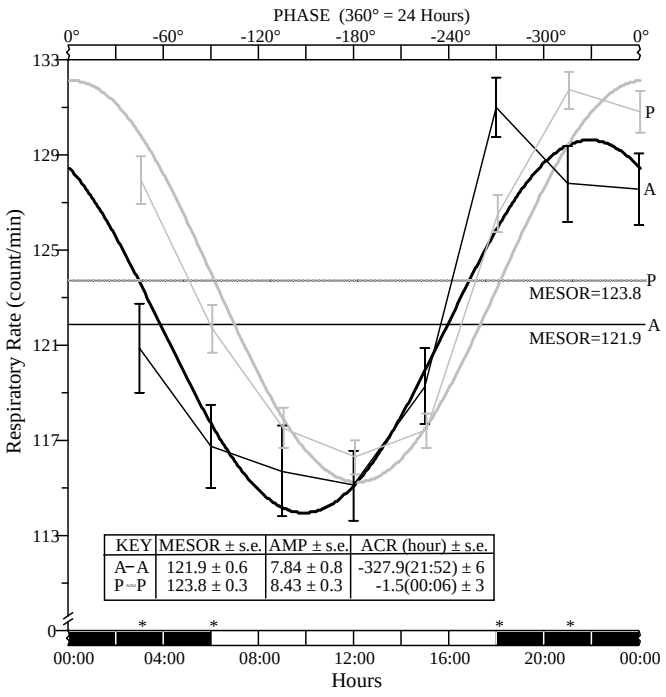


<Figure 2c. Heart Rate Comparison>



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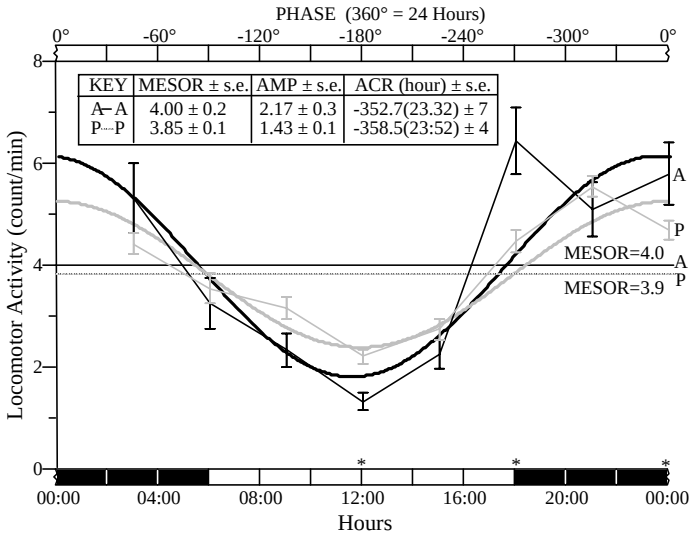

<Figure 2e. Locomotor Activity Comparison>

Fig. (2). Ambulatory monitoring of systolic and diastolic blood pressure, heart rate, respiratory rate and locomotor activity before and after L-NAME treatment. Radio-telemetered blood pressure, heart rate, respiratory rate and locomotor activity levels were measured every 4 min for 5 days before (A) treatment (Rx) and for 14 days after (P)-Rx. Every 4-min telemetered data were collected using the Data Quest Program (Data Science International, St. Paul, MN) [2] and converted into hourly averages, and analyzed by the linear least square rhythmometry [21]. A *p* value of less than 0.05 was considered significant. SBP: systolic blood pressure (Fig. **2a**); DBP: diastolic blood pressure (Fig. **2b**); HR: heart rate (Figure 2c); RR: respiratory rate ((Fig. **2d**); LA: locomotor Activity (Fig. **2e**); L-NAME: N-omega-nitro-L-arginine methyl ester. * $P < 0.05$.

Table **3** shows daily circadian SBP and HR monitoring statistics in L-NAME treatments from day-1 through day-14 compared with the baseline values. MESORS of SBP were increased, while those of HR were dramatically suppressed as adding days with L-NAME treatments. Significant swinging amplitudes in HR were noted during the first 6 days of L-NAME treatments, as compared to those of SBP with relatively stable amplitudes. The Acrophase was delayed in SBP from the baseline values with somewhat various changes in HR, especially first few days of treatments. In the study time point comparisons in Table **4**, there were no predictable changes at various time points, but SBP showed a greater tendency to change during the night time, with a markedly reduced HR at each time point around the clock (Table **4**).

Table 4. Circadian Variation of Systolic Blood Pressure and Heart Rate Before and After L-NAME Treatment

	Hours							
RxT (n=5)	**3:00 AM**	**6:00 AM**	**9:00 AM**	**Noon**	**3:00 PM**	**6:00 PM**	**9:00 PM**	**Midnight**
SBP (mm Hg)								
Basal *vs*.	143 ± 2	137 ± 2	136 ± 2	135 ± 1	139 ± 2	154 ± 1	151 ± 2	151 ± 2
Day 1	142 ± 6	137 ± 4	134 ± 3	136 ± 3	141 ± 2	151 ± 5	152 ± 3	148 ± 6
Day 2	155 ± 4†	146 ± 4*	142 ± 3	142 ± 3*	141 ± 3	154 ± 4	155 ± 2	155 ± 5
Day 3	139 ± 4	135 ± 4	132 ± 4	130 ± 4	138 ± 4	150 ± 4	142 ± 6*	145 ± 6
Day 4	161 ± 4‡	153 ± 3§	143 ± 4*	141 ± 2	139 ± 3	159 ± 5	160 ± 4*	163 ± 4*
Day 5	160 ± 5‡	154 ± 4¶	144 ± 4†	141 ± 2	144 ± 3	155 ± 4	158 ± 3	161 ± 3*
Day 6	155 ± 5*	147 ± 5*	140 ± 4	137 ± 4	143 ± 3	153 ± 3	161 ± 4*	160 ± 5*
Day 7	155 ± 6*	146 ± 5*	140 ± 5	137 ± 4	137 ± 4	150 ± 4	160 ± 4*	158 ± 5
Day 8	153 ± 5	148 ± 5*	142 ± 4	142 ± 4	138 ± 4	148 ± 5	159 ± 4	158 ± 5
Day 9	158 ± 3‡	152 ± 4¶	148 ± 3†	143 ± 3*	142 ± 4	148 ± 3	160 ± 4*	159 ± 3
Day 10	158 ± 4‡	150 ± 5‡	143 ± 4	141 ± 3	143 ± 3	149 ± 4	156 ± 4	157 ± 4
Day 11	157 ± 4†	145 ± 5	142 ± 4	140 ± 3	142 ± 4	150 ± 3	160 ± 3*	160 ± 4*
Day 12	156 ± 5†	150 ± 5‡	145 ± 3*	142 ± 3	142 ± 3	152 ± 4	159 ± 3	159 ± 3
Day 13	157 ± 5†	153 ± 5¶	146 ± 4*	141 ± 4	141 ± 3	151 ± 3	160 ± 4*	161 ± 3*
Day 14	150 ± 8	143 ± 7	138 ± 6	140 ± 4	145 ± 4	152 ± 4	162 ± 4*	159 ± 5
HR (Beat/min)								
Basal *vs*.	521 ± 08	497 ± 07	507 ± 07	510 ± 07	547 ± 07	635 ± 06	604 ± 08	591 ± 08
Day 1	490 ± 17	431 ± 18¶	429 ± 13¶	464 ± 12†	534 ± 28	615 ± 20	559 ± 14	551 ± 23
Day 2	615 ± 15¶	583 ± 17¶	587 ± 13¶	600 ± 08¶	533 ± 18	604 ± 15*	594 ± 16	582 ± 24
Day 3	459 ± 17‡	422 ± 21¶	425 ± 18¶	440 ± 14¶	552 ± 23	612 ± 12	547 ± 18‡	529 ± 20‡
Day 4	472 ± 16*	427 ± 22§	419 ± 20¶	417 ± 19¶	433 ± 18¶	578 ± 08¶	468 ± 09¶	479 ± 17¶
Day 5	449 ± 11‡	412 ± 22¶	409 ± 19¶	418 ± 13¶	424 ± 08¶	518 ± 19¶	502 ± 17¶	484 ± 12¶
Day 6	459 ± 18‡	418 ± 18¶	396 ± 16¶	432 ± 14¶	447 ± 16¶	516 ± 10¶	528 ± 16¶	476 ± 20¶
Day 7	441 ± 16¶	388 ± 14¶	424 ± 16¶	413 ± 16¶	428 ± 13¶	487 ± 13¶	542 ± 14‡	488 ± 14¶
Day 8	437 ± 19¶	394 ± 15¶	405 ± 21¶	437 ± 24¶	420 ± 14¶	485 ± 17¶	552± 13†	480 ± 17¶
Day 9	451 ± 15‡	389 ± 16¶	412 ± 23¶	421 ± 16¶	445 ± 11¶	493 ± 16¶	535 ± 20‡	474 ± 14¶
Day 10	471 ± 18*	395 ± 15¶	412 ± 25¶	435 ± 14¶	414 ± 16¶	490 ± 17¶	528 ± 15¶	491 ± 22¶
Day 11	457 ± 19‡	415 ± 17¶	433 ± 22¶	433 ± 18¶	452 ± 14¶	497 ± 15¶	546 ± 14‡	504 ± 11¶
Day 12	482 ± 22	442 ± 23‡	430 ± 20¶	429 ± 21¶	445 ±11¶	497 ± 16¶	555 ± 20*	522 ± 15¶
Day 13	483 ± 19	456 ± 17*	428 ± 19¶	423 ± 16¶	460 ± 18¶	500 ± 17¶	571 ± 13	523 ± 18§
Day 14	490 ± 26	458 ± 22*	451 ± 24‡	451 ± 16‡	490 ± 23‡	523 ± 17¶	576 ± 17	528 ± 15‡

RxT: L-NAME treatment time; SBP: systolic blood pressure; HR: heart rate.
*P < 0.05; †P < 0.01; ‡P < 0.005; §P < 0.001; ¶P < 0.0001

Fig. (**2**) shows not only overall circadian rhythms but also test-time point comparisons. SBP was significantly higher at midnight, 03:00, 06:00, 9:00, noon, 18:00, 21:00 and midnight hours (all $P < 0.05$), while there was no significant difference at 15:00 hour after L-NAME Rx (Fig. **2a**). DBP showed nearly the opposite results: A significant decrease at 15:00 and 18:00 hours ($P < 0.05$), while all other times showed no significant difference following L-NAME-Rx (Fig. **2b**). Significantly depleted HR was noted at all 8 time point comparisons after L-NAME treatment (Fig. **2c**), while RR was significantly elevated at 03:00 (Tables **2-4**), 6:00, 18:00 and 21:00 hours (all $P < 0.05$, Fig. **2d**). Overall MESOR of the LA was not significantly changed after L-NAME treatment. However, in time point comparisons, reduced activity was noted during the night time when the light was off at 18:00 hour through midnight (Fig. **2e**, $P < 0.0001$), while an opposite result was observed at noon, with significantly increased activity in this nocturnal animal ($P < 0.005$). The results suggest that an NOS blockade may impair cardiovascular autonomic adaptations and arterial

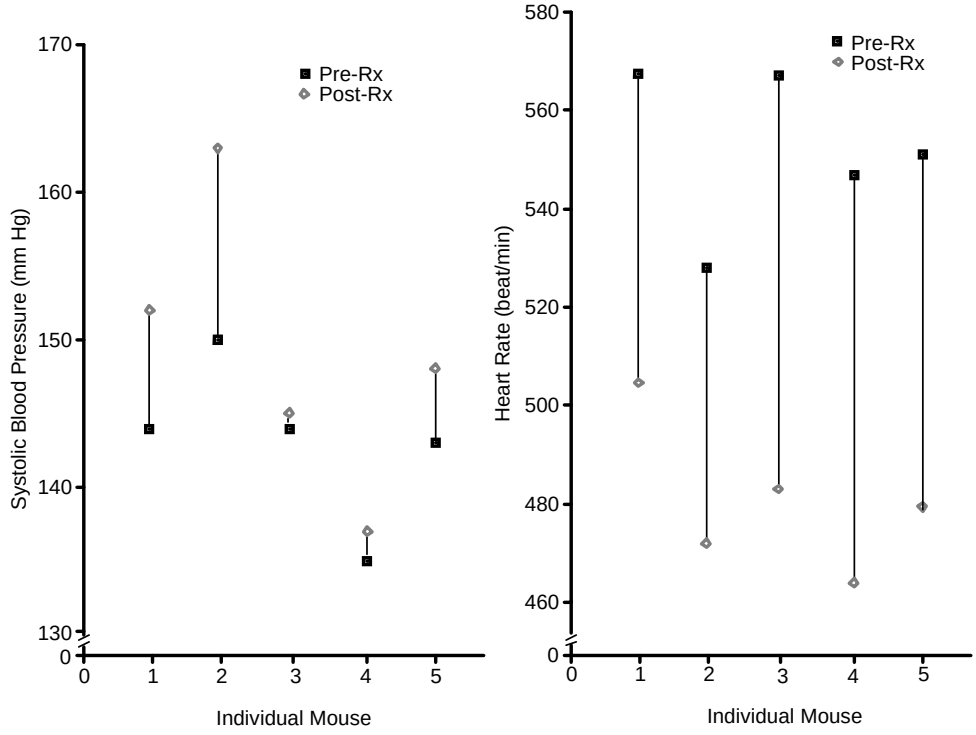

Fig. (3). Systolic Blood Pressure and Heart Rate Comparisons in Individual Mice before and after L-NAME Treatment. After L-NAME treatment, all individual mice had various degrees of increased systolic blood pressure with a markedly depleted heart rate. Rx: L-NAME treatment.

reflex integration, resulting in increased vascular tone during the systole, but not the end diastole in the relaxed cardiac autonomic tonus. In individual comparisons in SBP and HR, all 5 mice showed various levels of increased SBPs, while shockingly depleted HR in each individual mouse following L-NAME treatment, resulted in a slightly but a significantly increased group MESOR of SBP and a profound bradycardia, as shown in Tables **2-4** and Figs. (**2, 3**). The bradycardia started in the initial stage of the L-NAME treatment and stayed throughout the study span (Tables **3** and **4**).

DISCUSSION

NO is regulated in lung and tissue vasodilator and vasoconstrictor activities by a partition between NO, bound to heme iron and to the Cysb93 thiol of Hb, and acts as a principal regulator of vascular function [22]. Hemes sequester NO, while thiols destroy NO bioactivity. Decreased bioactive NO results in vasoconstriction and NO production is facilitated by a degree of hypoxia [23]. NO can react with sulfhydryl groups yielding nitrosothiols and upon decomposition, oxidize thiols. This was shown in our recent study, which demonstrated that the pretreatment of erythrocytes (RBC) with an SH-reducing agent reduces sodium permeability selectively in Caucasian RBC with hypertensive high NaCl permeability, but has no effect on normotensive low RBC NaCl permeability. This indicates that oxidized sulfhydryl groups (or nitrosothiols) in cell membrane proteins may be involved in patients with essential hypertension [24]. There may be racial differences that affect hypertension, such as a reduced endothelial release of bioactive NO shown in the large African American Heart Failure Trial. That study compared the anti-hypertensive effects of BiDil, an orally administered NO enhancing medicine (combined isosorbide dinitrate and hydralazine) demonstrated preferential effectiveness in blacks, which supports further the concept of reduced endothelial cell NO generation in blacks [25,26].

L-NAME inhibits NOS, including those beyond the blood brain barrier. The hypertension resulting from chronic L-NAME treatment may relate to the actions of NO in the vasculature, renal medulla, brainstem, or higher central nervous structures. Neuronal NOS mRNA expression was about 50% lower in the brain and kidney of salt-sensitive Dahl (DS) rats than in salt-resistant Dahl (DR) rats under all dietary conditions [27] and DS responded to a high NaCl diet with a dramatic increase in BP [3,16,17]. When treated with selective NOS inhibitors, such as 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT), S-ethylisourea (EIT) or N-[3-(aminomethyl)benzyl]acetamidine hydrochloride, SBP rose significantly in DR rats after 7 days of an 8% NaCl diet [28]. Moderately high fat diet-induced defects in NO production may promote the salt-sensitivity of BP in obese Zucker rats, which appear to require more NO to maintain BP during a salt challenge [29]. These results indicate that reduced NO production may be involved in salt-induced hypertension. Individuals with salt-sensitivity can arise from either hereditary or acquired defects in renal function. Sodium balance and the regulation of renal sodium excretion are a couple of the important homeostatic functions of the body in BP regulation. Genetic causes of salt sensitivity in single gene mutations are often manifested in a significant family history of hypertension, and variations in cellular Na^+/K^+-ATPase activity may play an important role in the regulation of ion transport and fluid balance, as well as the control of BP levels [30-34].

In the current study, before the L-NAME treatment, the baseline circadian values were established following completion of the 4 week monitoring to ensure a complete recovery with a resynchronized circadian rhythm from the telemetry surgery. We observed that only SBP, but not DBP, was elevated with the L-NAME treatment. The NOS blockade may impair cardiovascular autonomic adaptations, resulting in increased vascular tone during the systole, but not the end diastole in the relaxed cardiac autonomic tonus. SBP is mainly affected by changes in stroke volume and/or vascular compliance due to physical reasons, but only slightly by resistance, while DBP is mostly affected by resistance. A rise in SBP with unchanged DBP would be compatible with changes in stroke volume and compliance than with a rise in resistance.

L-NAME-treated mice became hypertensive with enhanced baroreflex and chemoreflex, while showing no differences in heart rates [15]. The enhancement of baroreflex sensitivity and attenuation of the Bezold-Jarisch cardiopulmonary reflex and chemoreflex seem to be mainly caused by the inhibition of NO synthesis. Although those studies involved direct measurements of mean arterial pressure and heart rate, they were under anesthesia [15], while our current study measured the telemetered status of fully recovered and stabilized test subjects. As predicted, their BP levels were comparable throughout our extensive direct measurements [1-3,16-18], while other activities, including HR, RR and LA comparisons may not be comparable under an anesthetized status. In our study, L-NAME treatment significantly reduced heart rate, 552.13 ± 2.7 *versus* 481 ± 1.8 (beat/min), with a shifted peak time and significantly reduced double amplitude. Our results were consistent at every tested time (Fig. **2**, Tables **3** and **4**) and in each individual mouse (Fig. **3**). Studies in clock gene expression and BP rhythms in humans and mice with NO-production are not new, as shown in NOS inhibition, which resulted in the impairment of clock gene expression and BP rhythms [19,35]. But some studies in L-NAME treated wild type mice showed increases in both SBPs and DBPs without affecting circadian rhythms in BP and HR [36], which can be seen in some of the experimental methodologies.

NO may impair baroreflex integration. The inhibitory influence of NO on the arterial baroreflex is present in fetal life. The gradient of the pulse interval-arterial BP relationship was nearly doubled during the NOS blockade in fetal sheep, demonstrating that NO attenuates the sensitivity of the cardiac baroreflex during fetal life and that NO exerts inhibitory influences on the integration of afferent discharge from the arterial baroreceptors [37]. Hypertension and bradycardia could either derive from the independent effects of LNAME on vascular tone and HR, from baroreflex interaction, or from a combination thereof.

We did not measure the catecholamine values, which should be included in a future study to examine variation changes in the sympathetic nervous system, as well as all organ systems on genetic and environmental bases. This will help explain the systemic effects of NOS inhibition in the central and autonomic nervous systems, affecting the final cardiovascular outcomes in genetic and epigenetic based hypertension.

In summary, L-NAME-Rx increased circadian SBP significantly, mainly during the night time points, and markedly reduced circadian HR at almost every time-point throughout the 24-h span (Tables **2**, **3** and **4**, Figs. **2a** and **2c**). Time-point depleted LA was noted, when the light was off at 18:00 hour through midnight following L-NAME-Rx. An opposite result was observed at noon, with a significantly increased LA in this nocturnal animal, and a markedly decreased amplitude (Fig. **2**, Table **2**). Interestingly, we observed markedly reduced HR with L-NAME-Rx, as compared with other reports. The measurements by telemetry of every 4-min for 14 days with L-NAME treatment provide a very solid result. It is remarkable that besides marked reductions in MESORs of HR, circadian variations at almost every time-point compared before and after Rx were significant (most $P < 0.0001$).

CONCLUSION

Ambulatory cardiovascular activities with L-NAME treatment have not been extensively studied. In the current study, we monitored ambulatory cardiovascular activities. SBP but not DBP was elevated with L-NAME treatment, and the animals showed less pronounced activity during their active period and remained less calm during their resting period, resulting in a smaller circadian rest/activity variation. Markedly depleted HRs were observed with L-NAME-treatment in the current study, while others reported elevated or unchanged HR. It should be noted that our results were from a longer-term ambulatory monitoring under stabilized physiological conditions, while other studies were under anesthesia. The recording of the activity index and the careful analysis of the nadir and peak rhythmicity should be a new addition to the ambulatory data collection. The study results should extend our understanding of cardiovascular physiology and the BP regulation in humans.

SOURCES OF FUNDING

This work was supported by the U.S. National Institutes of Health [RO1 HL-35643] and a Grant by the Minnesota Medical Foundation, Minneapolis, Minnesota.

ACKNOWLEDGEMENTS

The authors thank Mr. John S. Lee for his review, comments and editorial assistance.

CONFLICT OF INTEREST

None declared.

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Received: June 16, 2011 Revised: August 21, 2011 Accepted: September 12, 2011