Chemical sympathectomy restores baroreceptor-heart rate reflex and heart rate variability in rats with chronic nitric oxide deficiency

Meenakshi Chaswal[1] MD,DNB,PhD, Shobha Das[2] MD, Jagdish Prasad[3] MS,Mch, Anju Katyal[4] PhD, Mohammad Fahim[5] PhD.

Author Information

1 Department of Physiology, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India.

2 Formerly Director Prof. & Head, Department of Physiology, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India.

3 Department of Cardiothoracic & Vascular Surgery, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

4 Department of Biochemistry, B R Ambedkar Centre for Biomedical Research, University of Delhi, India

5 Department of Physiology, Hamdard Institute of Medical Sciences & Research, Jamia Hamdard, New Delhi, India

Correspondence to: Dr Mohammad Fahim, Ph.D., Head, Department of Physiology, Hamdard Institute of Medical Sciences & Research, Jamia Hamdard, New Delhi, India.
E-mail: vpciphysiology@yahoo.com Phone: 09899218021.

Short running title

Sympathoexcitation mediated autonomic dysfunction in L-NAME hypertension.

Summary

Nitric oxide (NO) plays a crucial role not only in regulation of blood pressure but also in maintenance of cardiac autonomic tone and its deficiency induced hypertension is accompanied by cardiac autonomic dysfunction. However, underlying mechanisms are not clearly defined. We hypothesized that sympathetic activation mediates hemodynamic and cardiac autonomic changes consequent to deficient NO synthesis. We used chemical sympathectomy by 6-hydroxydopamine to examine the influence of sympathetic innervation on baroreflex sensitivity (BRS) and heart rate variability (HRV) of chronic N^G–nitro-L-arginine methyl ester (L-NAME) treated adult Wistar rats. BRS was determined from heart rate responses to changes in systolic arterial pressure achieved by intravenous administration of phenylephrine and sodium nitroprusside. Time and frequency domain measures of HRV were calculated from 5-minute electrocardiogram recordings. Chronic L-NAME administration (50 mg/kg per day for 7 days orally through gavage) in control rats produced significant elevation of blood pressure, tachycardia, attenuation of BRS for bradycardia and tachycardia reflex and fall in time as well as frequency domain parameters of HRV. Sympathectomy completely abolished the pressor as well as tachycardic effect of chronic L-NAME. In addition, BRS and HRV improved after removal of sympathetic influence in chronic L-NAME treated rats. These results support the concept that an exaggerated sympathetic activity is the principal mechanism of chronic L-NAME hypertension and associated autonomic dysfunction.

Key words

Nitric oxide • N^G–nitro-L-arginine methyl ester • Sympathectomy • 6-hydroxydopamine • Autonomic dysfunction

Introduction

Nitric oxide (NO) generated from L-arginine by the action of the enzyme nitric oxide synthase (NOS) plays an important role in basal and dynamic regulation of circulation (Gladwin *et al*. 2004). It has been firmly established that pharmacological inhibition of NO synthesis produces acute and chronic hypertension in many animal species (Rees *et al*. 1989, Aisaka *et al*. 1989), but the underlying mechanisms mediating the rise in pressure are incompletely understood. Although this pressor response at first was attributed solely to inhibition of tonically produced vasodilatory NO (Rees *et al*. 1989, Aisaka *et al*. 1989), an emerging body of literature suggests the possible role of sympathetic nervous system (SNS), renin–angiotensin-aldosterone system and of the oxidative stress (Bernatova 2014), however the results are inconclusive. The existence of sympathoexcitatory state after NOS inhibition is quite possible as NO also modulates autonomic control of cardiovascular system by tonically inhibiting sympathetic outflow and increasing parasympathetic influence (Schultz 2009). However, the role of sympathetic neural control during pharmacological inhibition of NO by inhibitors such as L-NAME remains ill defined. Few studies reported enhanced sympathetic flow in L-NAME hypertension (Biancardi *et al*. 2007, Young *et al*. 2009, Chaswal *et al*. 2011, Augustyniak *et al*. 2006) whereas others showed no association between the two (Dos Santos *et al*. 2010, Ramchandra *et al*. 2007). Although involvement of sympathetic innervation in L-NAME hypertensive model has been addressed in earlier studies but to the best of our knowledge, there is absence of a comprehensive study which has examined alterations in cardiac autonomic functions after L-NAME treatment and influence of sympathetic tone on this. If NO inhibition is characterized by sympathetic over activity then removal of sympathetic influence should restore cardiac autonomic alterations following L-NAME treatment. Therefore, in order to clarify the

role of sympathetic innervation in hemodynamic and cardiac autonomic changes resulting after NO synthesis inhibition, we measured noninvasive markers of cardiac autonomic control such as baroreflex sensitivity and heart rate variability after chronic L-NAME treatment in rats with or without sympathectomy.

Materials and methods

Animals

40 adult male Wistar rats weighing between 250-300 g were used. Rats were housed in polyethylene cages with a controlled temperature (25 ± 2^{o}C) and a 12:12-hour dark-light cycle. They were allowed food and water ad libitum.

The experimental protocol was approved by Institutional Animal Ethical Committee of B R Ambedkar Centre for Biomedical Research, Delhi, India. All experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Sympathectomy was obtained by 6-OHDA (100 mg/kg) administered two times over a 1-week period. The drug was dissolved in 0.9% w/v NaCl solution containing 1% ascorbic acid and was injected intraperitonealy. The adequacy of sympathectomy was verified by the drastic attenuation of the pressor and tachycardic responses to tyramine (100 µg/kg, i.v.) (Mircoli *et al*. 2002).

Control rats were injected vehicle alone. 24 hrs after sympathectomy or vehicle injection, rats received daily L-NAME 50 mg/kg dissolved in water or only water administered through gavage for 7days. Thus our study had four groups as follows:

Group I (C): (n=10) Control rats with intact sympathetic nerve supply.

Group II (C L): (n= 10) Chronic L-NAME treated control rats.

Group III (S): (n= 10) Sympathectomised rats.

Group IV (SL): (n= 10) Chronic L-NAME treated sympathectomised rats.

Surgical preparation of animals

Rats were anaesthetized with urethane (1000mg/kg, i.p.) and placed on a heated surgical table so as to maintain body temperature at around 37^{o}C. Tracheostomy was performed and animals were allowed to breath spontaneously through tracheal cannula. After tracheal cannulation, femoral artery was cannulated and arterial blood pressure (ABP) recorded by pressure transducer (Statham-P23D). The femoral vein was also cannulated for drug administration. Prior to recording ABP, pressure recording system was calibrated with the help of a mercury manometer. ABP was measured after 20 min of stabilization period.

ABP and heart rate (HR) were recorded on a Power Lab data-acquisition system (4SP, AD Instruments, Australia) with a computerized analysis programme (Chart 5.4.2, AD Instruments, Australia).

Measurement of baroreflex sensitivity (BRS)

BRS was measured by administering phenylepherine (20µg/ml/kg) and sodium nitroprusside (20µg/ml/kg) through venous catheter. Each drug was injected i.v. as bolus dose to cause respectively rise and fall in ABP. Injections were separated by 15 min interval to allow the parameters to stabilize. The relationship between rise in systolic blood pressure (SBP) evoked by phenylephrine and associated bradycardia or fall in SBP evoked by sodium nitroprusside and associated tachycardia was assessed by regression analysis for individual animal. The regression

coefficient (slope of regression line) expressed as beats per minute per mm of mercury (bpm/mmHg), was taken as an index of BRS.

Electrocardiogram (ECG) recording

Bipolar limb lead II was used for recording ECG. Electrodes were placed subcutaneously and were connected through a bioamplifier (AD instruments, Australia) to the Power Lab data-acquisition system.

Estimation of Heart rate variability (HRV)

We assessed heart rate variability by time domain as well as frequency domain (spectral) analysis of each of 5-minute ECG recording using HRV software (AD Instruments, Australia). Parameter used for time domain analysis was SDNN i.e. standard deviation of normal R-R intervals. It is expressed in milliseconds (ms) and provides a measure of the total variability of heart rate. The other time domain parameter was square root of the mean squared differences of successive R-R intervals (RMSSD). RMSSD estimates parasympathetic drive of autonomic nervous system. Power spectral analysis of heart rate variability was performed by fast-Fourier transformation technique. Frequency bands defined for spectral analysis were: total spectral power(P) from 0 to 3 Hz, low frequency (LF) band from 0.25 to 1Hz and high frequency(HF) band from 1to 3Hz (corresponding to the observed respiratory frequency of animals). The frequency ranges chosen were based on data from previous report (Baumert *et al*. 2007). Spectral power in different frequency bands is expressed in ms^2. The LF to HF ratio (LF: HF) was calculated to estimate the sympatho-vagal balance.

Chemicals

Chemicals employed in the experimental protocol were L-NAME, 6- hydroxydopamine, phenylephrine hydrochloride, sodium nitroprusside, and urethane. All chemicals used in this study were of analytical grade and were obtained from Sigma-Aldrich (USA) and Merck.

Statistical analysis

The results are presented as means ± S.E.M. Statistical significance was calculated from Kruskal- Wallis test with post-hoc Dunn's test. The value was considered significant at $p<0.05$. Statistical analysis was performed using the Statistical Analysis Systems (SAS) software.

Results

Effect of L-NAME on hemodynamic responses of control and sympathectomised rats

Table 1 shows cardiovascular data after chronic L-NAME treatment of control and sympathectomised rats. Baseline blood pressure and heart rate were similar in control and sympathectomised rats. After chronic L-NAME treatment of control rats a significant rise in blood pressure and heart rate was observed but in sympathectomised rats the pressor and tachycardic effect of L-NAME was not seen. As a result, a significant difference in heart rate and blood pressure was observed in L-NAME treated sympathectomised rats in comparison to L-NAME treated control ones.

Baroreflex responses following L-NAME in control and sympathectomised rats

BRS for bradycardia reflex (Fig. 1A) consequent to rise in pressure by phenylepherine was similar in control and sympathectommised rats (2.0 ± 0.2 bpm/mmHg versus 2.0 ± 0.1 bpm/mmHg, ns). Chronic L-NAME treatment attenuated BRS for bradycardia reflex in control rats (0.8 ± 0.04 bpm/mmHg versus 2.0 ± 0.2 bpm/mmHg, $p<0.05$) but not in sympathectomised

rats (1.7 ± 0.1 bpm/mmHg versus 2.0 ± 0.1 bpm/mmHg, ns). Therefore, L-NAME associated diminished BRS for bradycardia reflex was normalized after sympathectomy (Fig. 1A).

Baseline BRS for tachycardia reflex (Fig. 1B) was lower in sympathectomised rats compared to control animals (0.7 ± 0.1 bpm/ mmHg versus 1.4 ± 0.09 bpm/mmHg, $p<0.05$). A marked suppression of BRS was seen after 7 days of L-NAME administration in control animals (0.7 ± 0.05 bpm/mmHg versus 1.4 ± 0.09 bpm/mmHg, $p<0.05$). BRS for tachycardia response remained low after L-NAME administration in sympathectomised rats (0.9 ± 0.06 bpm/mmHg versus 0.7 ± 0.1 bpm/mmHg, ns).

Effect of L-NAME on HRV of control and sympathectomised rats

Figure 2 demonstrates time domain parameters of HRV. Baseline SDNN (Fig. 2A) was similar in control and sympathectomised rats (4.1± 0.2 ms versus 3.6 ± 0.2 ms, ns). A significant fall of SDNN was seen following L-NAME treatment in control (2.2 ± 0.1 ms versus 4.1 ± 0.2 ms, $p<0.05$) but not in sympathectomised rats (3.9 ± 0.2 ms versus 3.6 ± 0.2 ms, ns).

RMSSD (Fig. 2B) showed similar trend with no difference between control and sympathectomised rats (4.3 ± 0.2 ms versus 3.9 ± 0.1 ms, ns). L-NAME administration in control rats significantly lowered RMSSD values (1.7 ± 0.1 ms versus 4.3 ± 0.2 ms, $p<0.05$) but this fall was not seen after L-NAME administration in sympathectomised animals (3.6 ± 0.2 ms versus 3.9 ± 0.1 ms, ns). RMSSD was also significantly lower in L-NAME treated control rats compared to L-NAME treated sympathectomised rats (1.7 ± 0.1 ms versus 3.6 ± 0.2 ms, $p<0.05$).

Figure 3 is original tracing of record showing power spectral density of HRV with or without L-NAME in control and sympathectomised animals. Baseline total spectral power of HRV (Fig. 4 A) did not vary between control and sympathectomised rats (10.1 ± 0.9 ms^2 versus

9.6 ± 0.9 ms^2, ns). L-NAME treatment in control rats produced a significant fall in total power (2.7 ± 0.3 ms^2 versus 10.1 ± 0.9 ms^2, $p<0.05$). However no difference was seen after L-NAME in sympathectomised rats (9.4 ± 0.6 ms^2 versus 9.6 ± 0.9 ms^2, ns). Comparison of total power after L-NAME in control and sympathectomised rats showed a significant difference ($p<0.05$).

Low frequency spectral power of HRV (Fig. 4B) showed no variation between control and sympathectomised rats (1.1± 0.1 ms^2 versus 0.9 ± 0.1 ms^2, ns). Reduced LF spectral power was observed after L-NAME in control rats (0.4 ± 0.04 ms^2 versus 1.1 ± 0.1 ms^2, $p< 0.05$) but no such difference was observed in sympathectomised rats (0.8 ± 0.1 ms^2 versus 0.9 ± 0.1 ms^2, ns).

HF spectral power (Fig. 4C) of control rats was similar to sympathectomised animals (5.6 ± 0.7 ms^2 versus 4.5 ± 0.3 ms^2, ns). HF spectral power decreased significantly following L-NAME in control rats (1.0 ± 0.1 ms^2 versus 5.6 ± 0.7 ms^2, $p<0.05$) but no change in HF spectral power resulted after chronic administration of L-NAME in sympathectomised rats (4.0 ± 0.3 ms^2 versus 4.5 ± 0.3 ms^2, ns). HF power was significantly low after L-NAME in control rats compared to that in sympathectomised rats ($p<0.05$). LF to HF ratio (Fig. 4D) did not differ between control and sympathectomised rats (ns). LF to HF ratio increased significantly following chronic L-NAME treatment in control rats (0.4 ± 0.05 versus 0.2 ± 0.01, $p<0.05$) but no change was observed after L-NAME treatment of sympathectomised rats (0.2 ± 0.01 versus 0.2 ± 0.04, ns).

Discussion

Nitric oxide is said to buffer cardiac autonomic balance by restraining sympathetic and facilitating parasympathetic outflow (Schultz 2009). Deficiency of NO in addition to elevating blood pressure is thought to produce a dysfunctional state of autonomic balance with sympathetic activation. Although concept of sympathoexcitation in NO deficiency induced hypertension has been reported by previous studies but contradictory results make it difficult for a firm conclusion to be drawn (Biancardi *et al*. 2007, Young *et al*. 2009, Chaswal *et al*. 2011, Dos Santos *et al*. 2010, Ramchandra *et al*. 2007). Our study using chemical sympathectomy by 6-OHDA provides an indirect evidence of a state of sympathetic activation and its antagonistic influence in chronic L-NAME induced hypertension. The study shows that long term L-NAME treatment in control rats with intact sympathetic innervation resulted in a marked rise of blood pressure accompanied by tachycardia, attenuation of BRS for both bradycardia as well as tachycardia response, besides a significant decline in time and frequency domain parameters of HRV. Furthermore, sympathectomy by 6-OHDA reversed not only the cardiac autonomic dysfunction but also the hypertensive effect of L-NAME.

The present study was conducted on urethane anesthetized rats. Though in case of anesthetized preparation there may be interference of autonomic functions. However, in our study urethane has proved to be better than other anesthetics. Urethane is characterized by fairly good preservation of cardiovascular reflex responses and it minimally interferes with the physiological relevance of data collected in the anesthetized animals (Maggi & Meli 1986,).

Demonstration of pressor response following chronic administration of L-NAME is an expected finding and is in agreement with earlier reports (Biancardi *et al*. 2007, Serogin *et al*. 1998). However, the precise mechanism of L-NAME induced pressure rise remains ill defined.

Our study showed that chemical sympathectomy by 6-OHDA completely reversed the pressure rise seen after chronic L-NAME treatment, thus suggesting the existence of an important influence of sympathetic nervous system in mediating vascular effects of L-NAME. Our results are in agreement to a study by Sander *et al*. (1997) who found more than 50% attenuation of hypertensive response to 6 days of continuous L-NAME (50mg/Kg/day) administration by guanethidine induced sympathectomy. However, on literature survey we came across studies which contradict our findings as the one by Lepori *et al*. (1999) who reported potentiation of vasoconstrictor effect of NO synthesis inhibition by sympathectomy. This study was conducted on humans who had undergone thoracic sympathectomy for hyperhydrosis and an infusion of L-NMMA was used for blocking NO synthesis. The difference in species and methodology may explain divergent results.

Baroreceptor dysfunction may also contribute to pressor response of L-NAME. In our study presence of tachycardia despite elevation of blood pressure by L-NAME points to impairment of baroreceptor function, which was evident by attenuation of BRS for both bradycardia and tachycardia response in L-NAME treated rats. As impaired BRS for bradycardia response was normalised after sympathectomy, it reflects an important role of sympathetic activation rather than the structural alteration of vessels in decreasing BRS following L-NAME treatment. In agreement to our finding, sympathetic activation mediated baroreceptor impairment has also been reported by an earlier study (Ferrari *et al*. 1991). Though the reason for negative effect of sympathetic nervous system on baroreceptor –heart rate reflex is not investigated by our study but we may speculate that sympathetic over activity may be associated with increased tone of vessels such as carotid artery, reducing arterial distensibility and thus afferent discharge of baroreceptors (Lacolley *et al*. 1995, Mangoni *et al*. 1997). On removal of sympathetic influence,

the arterial tone gets normalized and thus BRS is restored. However sympathectomy in our study normalized only BRS for bradycardia response and we found decreased rather than preserved BRS for tachycardia response in L-NAME treated sympathectomised rats. This finding may reflect not only reduced sympathetic stimulation but also decreased parasympathetic withdrawal in chronic L-NAME treated sympathectomised rats.

In addition to impaired BRS, L-NAME treated hypertensive rats of our study also showed decrement of time and frequency domain parameters of HRV along with a significant rise of LF to HF ratio. Fall of RMSSD and HF spectral power of HRV, both of which are measures of parasympathetic tone and resting tachycardia suggest depression of cardiac vagal tone and predominance of sympathetic tone in L-NAME treated rats. As expected, reduction of cardiac vagal control was associated with a fall in SDNN, a measure of total variability along with reduced total spectral power. Another interesting finding was a reduction in LF spectral power following L-NAME treatment. Our results of HRV are in agreement to an earlier study by Souza *et al*. (2001) who also showed a fall in SDNN and LF spectral power in chronic L-NAME treated rats. Observation of decreased LF power in presence of sympathetic over activity is quite paradoxical, but as LF spectral power of HRV represents baroreflex related heart rate fluctuation therefore impaired BRS seen in L-NAME treated rats is likely to be associated with a reduced LF power (Van de Borne *et al*. 1997). Normalization of SDNN, RMSSD, total spectral power and also of HF spectral power after sympathetic blockade in L-NAME treated rats of our study further strengthens the concept of sympathetic over activity mediated autonomic alterations in L-NAME treated animals.

Even though our results of sympathectomy provide only an indirect indication of state of sympathoexcitation in chronic L-NAME treated animals, but there is evidence that L-NAME

when administered orally crosses blood brain barrier and causes inhibition of neuronal NOS, increasing central sympathetic outflow (Eshima *et al*. 2000). As explained earlier the enhanced sympathetic outflow impairs baroreceptor activity which in turn further potentiates the sympathoexcitatory response to NO inhibition.

In conclusion, our findings support the concept of increased sympathetic tone as an important pathogenic factor contributing to pressor response, impaired baroreflex sensitivity and reduced heart rate variability in L-NAME induced hypertension.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Authors thankfully acknowledge the support provided by Vardhaman Mahavir Medical College & Safdarjung Hospital, Department of physiology V.P. Chest Institute and BR Ambedkar Centre for Biomedical Research.

References

AISAKA K, GROSS SS, GRIFFITH OW, LEVI R: N^G – methylarginine, an inhibitor of endothelium derived nitric oxide synthesis, is a potent pressor agent in the guinea pig: does nitric oxide regulate blood pressure in vivo? *Biochem Biophys Res Commun* **160**: 881-886, 1989.

AUGUSTYNIAK RA, VICTOR RG, MORGAN DA, ZHANG W: L-NAME- and ADMA-induced sympathetic neural activation in conscious rats. *Am J Physiol Regul Integr Comp Physiol* **290**: R726-732, 2006.

BAUMERT M, NALIVAIKO E, ABBOTT D: Effect of vagal blockade on complexity of heart rate variability in rats. In: Kramar T J P, Zupanic A (eds.) IFBME Proceedings, 2007, pp 26- 29.

BERNATOVA I: Endothelial dysfunction in experimental models of arterial hypertension: cause or consequence? *Biomed Res Int* **2014**: 598271, 2014.

BIANCARDI VC, BERGAMASCHI CT, LOPES OU, CAMPOS RR: Sympathetic activation in rats with L-NAME-induced hypertension. *Braz J Med Biol Res* **40**: 401–408, 2007.

CHASWAL M, DAS S, PRASAD J, KATYAL A, FAHIM M: Cardiac autonomic function in acutely nitric oxide deficient hypertensive rats: role of the sympathetic nervous system and oxidative stress. *Can J Physiol Pharmacol* **89**:865-874, 2011.

DOS SANTOS FM, MARTINS DIAS DP, DA SILVA CAA, FAZAN R JR, SALGADO HC: Sympathetic activity is not increased in L-NAME hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* **298**: R89-R95, 2010.

ESHIMA K, HIROOKA Y, SHIGEMATSU H, MATSUO I, KOIKE G, SAKAI K, TAKESHITA A: Angiotensin in the nucleus tractus solitarii contributes to neurogenic hypertension caused by chronic nitric oxide synthase inhibition. *Hypertension* **36**: 259-263, 2000.

FERRARI AU, DAFFONCHIO A, FRANZELLI C, MANCIA G: Potentiation of the baroreceptor–heart rate reflex by sympathectomy in conscious rats. *Hypertension* **18**: 230–235, 1991.

GLADWIN MT, CRAWFORD JH, PATEL RP: The biochemistry of nitric oxide, nitrite and hemoglobin: role in blood flow regulation. *Free Radic Biol Med* **36**: 707-717, 2004.

LACOLLEY P, GLASER E, CHALLANDE P: Structural changes and in situ aortic pressure-diameter relationship in long-term chemical sympathectomized rats. *Am J Physiol* **269**: H407–H416, 1995.

LEPORI M, SARTORI C, DUPLAIN H, NICOD P, SCHERRER U: Sympathectomy potentiates the vasoconstrictor response to nitric oxide synthase inhibition in humans. *Cardiovasc Res* **43**: 739- 743, 1999.

MANGONI AA, MIRCOLI L, GIANNATTASIO C: Effect of sympathectomy on mechanical properties of common carotid and femoral arteries. *Hypertension* **30**: 1085–1088, 1997.

MIRCOLI L, FEDELE L, BENETTI M, BOLLA, GB, RADAELLI A, PERLINI S, FERRARI AU: Preservation of the baroreceptor heart rate reflex by chemical sympathectomy in experimental heart failure. *Circulation* **106**: 866–872, 2002.

RAMCHANDRA R, BARRET CJ, GUILD SJ, MCBRYDE F, MALPAS SC: Role of renal sympathetic nerve activity in hypertension induced by chronic nitric oxide inhibition. *Am J Physiol Regul Integr Comp Physiol* **292**: R1479–R1485, 2007.

REES DD, PALMER RMJ, MONCADA S: Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* **86**: 3375-3378, 1989.

SANDER M, HANSEN PG, VICTOR RG: The sympathetic nervous system is involved in the maintenance but not initiation of the hypertension induced by N (omega)-nitro-L-arginine methyl ester. *Hypertension* **30**: 64-70, 1997.

SEROGIN KE, HATTON DC, CHI Y, LUFT FC: Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system. *Am J Physiol Regul Integr Comp Physiol* **274** : R367-R374, 1998.

SCHULTZ HD: Nitric oxide regulation of autonomic function in heart failure. *Curr Heart Fail Rep* **6**: 71–80, 2009.

SOUZA HCD, BALLEJO G, SALGADO MC, DA SILVA VJ, SALGADO HC: Cardiac sympathetic overactivity and decreased baroreflex sensitivity in L-NAME hypertensive rats. *Am J Physiol Heart Circ Physiol* **280**: H844– H850, 2001.

VAN DE BORNE P, MONTANO N, PAGANI M, OREN R, SOMERS VK: Absence of low-frequency variability of sympathetic nerve activity in severe heart failure. *Circulation* **95**: 1449–1454, 1997.

YOUNG CN, FISHER JP, GALLAGHER KM, WHALEY-CONNELL A, CHAUDHARY K, VICTOR RG, THOMAS GD, FADEL PJ: Inhibition of nitric oxide synthase evokes central sympatho-excitation in healthy humans. *J Physiol* **587**: 4977-4986, 2009.

Table 1. Chronic L-NAME induced hemodynamic responses in control and sympathectomised rats.

Parameter	Control (n=10)	CL(n=10)	S (n=10)	SL(n=10)
SBP (mmHg)	112.4 ± 1.2	174.3 ± 5.5*	106.1 ± 1.7†	116.6 ± 1.7†
DBP (mmHg)	64.9 ± 2.9	117.9 ± 4.7*	59.4 ± 2.2†	68.5 ± 1.6†
MAP (mmHg)	81.5 ± 1.6	136.7 ± 4.9*	71.8 ± 3.2†	85.0 ± 1.6†
HR (bpm)	315.2 ± 11.4	371.2 ± 8.0*	357.7 ± 9.3	310 ± 7.1†‡

Control, rats with intact sympathetic nervous system; S, sympathectomised rats; CL, control rats treated with chronic L-NAME; SL, sympathectomised rats treated with chronic L-NAME; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; n, number of animals; bpm, beats per minute. Values are means ± S.E.M. * $p<0.05$ versus control; † $p< 0.05$ versus CL; ‡ $p<0.05$ versus S.

Legends of figures

Fig. 1. Baroreflex sensitivity (BRS) measured as ratio of (A) bradycardia response to rise in pressure by phenylephrine (B) tachycardia response to fall in pressure by sodium nitroprusside in each study group. C, Control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p<0.05$ versus CL.

Fig. 2. Bar graphs depicting (A) Standard deviation of normal R-R intervals (SDNN) and (B) Root mean squared difference of successive R-R intervals (RMSSD) in different groups. C, control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p<0.05$ versus CL.

Fig. 3. Power Spectral density of heart rate with or without L-NAME in control and sympathectomised rats (A) control rats (B) control rats treated with chronic L-NAME; (C) sympathectomised rats and (D) sympathectomised rats treated with chronic L-NAME.

Fig. 4. (A) Total spectral power; (B) low frequency spectral power; (C) high frequency spectral power and (D) LF to HF ratio (LF/HF) in each study group. C, Control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME; LF, low frequency; HF, high frequency. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p < 0.05$ versus CL.

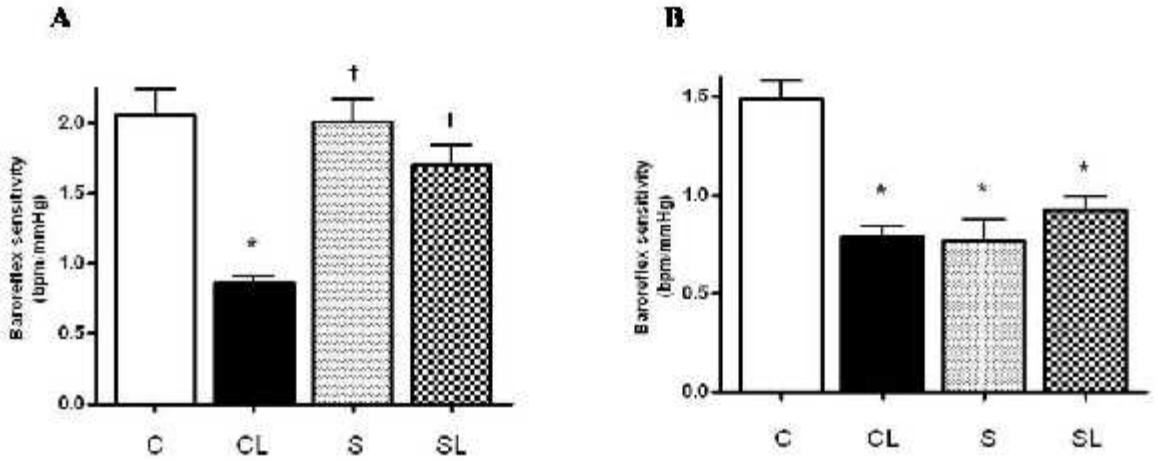

FIGURE 1

Fig. 1. Baroreflex sensitivity (BRS) measured as ratio of (A) bradycardia response to rise in pressure by phenylephrine (B) tachycardia response to fall in pressure by sodium nitroprusside in each study group. C, Control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p<0.05$ versus CL.

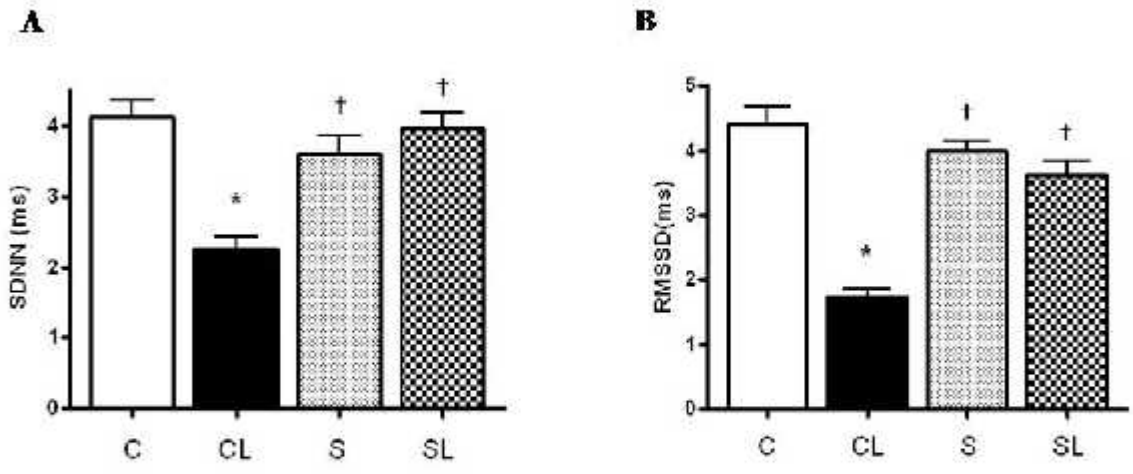

FIGURE 2

Fig. 2. Bar graphs depicting (A) Standard deviation of normal R-R intervals (SDNN) and (B) Root mean squared difference of successive R-R intervals (RMSSD) in different groups. C, control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p<0.05$ versus CL.

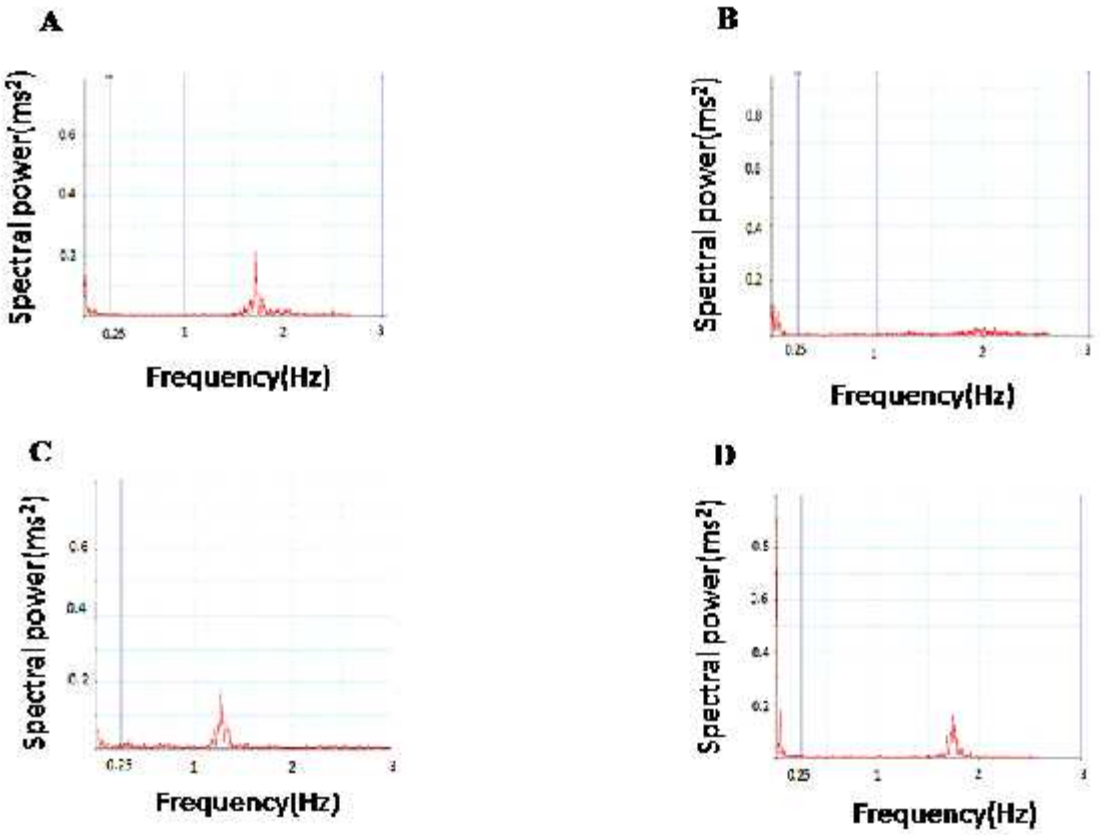

FIGURE 3

Fig. 3. Power Spectral density of heart rate with or without L-NAME in control and sympathectomised rats (A) control rats (B) control rats treated with chronic L-NAME; (C) sympathectomised rats and (D) sympathectomised rats treated with chronic L-NAME.

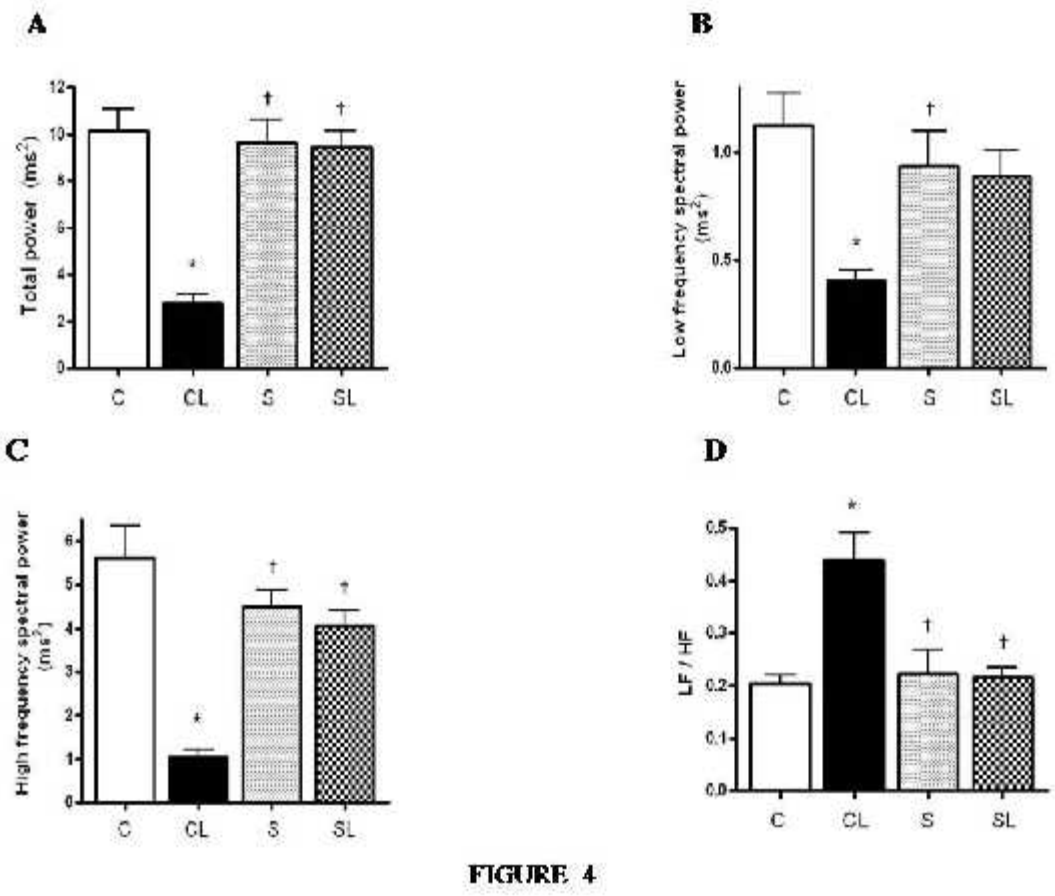

FIGURE 4

Fig. 4. (A) Total spectral power; (B) low frequency spectral power; (C) high frequency spectral power and (D) LF to HF ratio (LF/HF) in each study group. C, Control, rats with intact sympathetic nervous system; CL, control rats treated with chronic L-NAME; S, sympathectomised rats; SL, sympathectomised rats treated with chronic L-NAME; LF, low frequency; HF, high frequency. Values are means ± S.E.M. (n = 10). * $p<0.05$ versus Control; † $p < 0.05$ versus CL.