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Acute nitric oxide synthase inhibition and cardiac conduction in persons with spinal cord injury: a short report

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N^G-nitro L-arginine methyl ester (L-NAME) is a potent and non-specific inhibitor of nitric oxide synthase (NOS). NOS inhibition with L-NAME has been shown to adversely prolong electrocardiogram (ECG) intervals in an animal model, an observation which has yet to be evaluated in humans. We determined the effects of several weight-based L-NAME doses on ECG intervals in persons with tetraplegia and a neurologically-intact control group. This two-part investigation determined the effects of different weight-based doses of L-NAME in the supine (Study 1) and orthostatic position (Study 2). Subjects completed an open-label trial with intravenous administration of L-NAME at specific doses [i.e., 0, 1, 2 or 4 mg·kg^{-1}] in the supine position. The SCI group completed an orthostatic challenge with or without L-NAME [i.e., 0, 1 or 2 mg·kg^{-1}] and controls completed only a single visit [0 mg·kg^{-1}]. Digital ECGs were obtained at baseline (BL), after infusion (60 minutes) and 1 hour post-infusion (120 minutes) in Study 1, and at BL, 60 minutes and at two, 10 minute post-infusion time points after head up tilt (Post-Tilt 1 and 2) in Study 2. Heart rate, PQ, QT, and heart rate corrected QT (QTC) intervals were determined. The groups were matched for demographics. Seven subjects with tetraplegia and 6 controls participated in Study 1; 7 subjects with tetraplegia and 7 controls participated in Study 2. No statistical differences were noted between or within groups at baseline on each study visit for the ECG variables. L-NAME, regardless of dose, did not significantly change any ECG interval. NOS inhibition with L-NAME, at the weight-based doses tested do not induce hypertensive crises and, did not adversely affect any ECG interval in persons with SCI or neurologically intact control subjects during supine rest or orthostatic provocation.

1. Introduction

N^G-nitro L-arginine methyl ester (L-NAME) has been identified as a potent and non-specific inhibitor of all three nitric oxide (NO) synthase (NOS) isoforms including those of the inducible (iNOS), endothelial (eNOS) and neuronal (nNOS) isoforms (Rees et al. 1989; Sander et al. 1999). L-NAME has been used experimentally as a hypertensive agent in humans (Wecht et al. 2007, 2008, 2011) and in elucidating the role of NO in cardiac electrophysiology in various animal models (Neunteufl et al. 2000; Sankatsing et al. 2005; Fellet et al. 2006), with specific evidence of significant prolongation of the PR interval (Lenaerts et al. 2011). NO is synthesized *in vivo* from the amino acid L-arginine and oxygen by NOS (Moncada and Higgs 1993; Vallance and Moncada 1994; Chowdhary and Townend 1999). NO that is derived from nNOS accentuates vagal cardiomotor effects, especially dromotropic actions (e.g., atrioventricular conduction duration), and it is speculated to be a modulator of vagal chronotropic responses (Clarkson et al. 1996; Ellestad 1996; Elvan et al. 1997; Schwartz 1998; Straus et al. 2006). From a mechanistic perspective, nNOS transcription and activity appears to be regulated, in part, by calcium (Ca^{2+}), (Barouch et al. 2002; Oceandy et al. 2007; Xu et al. 1999) which is central to phase 2 of cardiac action potentials (Niedergerke and Orkand 1966). The role of sympathetic neuromodulation on cardiac inotropy and chronotropy is well appreciated.

Norepinephrine, a sympathetic neurotransmitter, mediates cardiac ionotropic, chronotropic, and lusitropic effects through ionic mechanisms, including Ca^{2+} homeostasis (Steinberg 1999). Maintenance of appropriate cytosolic Ca^{2+} concentration is crucial for cardiac electrical stability (Golden et al. 2000) and activation of the adrenergic receptors alters the expression of genes that encode cardiac Ca^{2+} regulatory proteins (Linck et al. 1998; Golden et al. 2001). In cervical lesion spinal cord injury (i.e., tetraplegia), it is generally accepted that cardiac sympathetic tone is grossly attenuated or otherwise ablated following injury (Inoue et al. 1995; Grimm et al. 1997; Wang et al. 2000). On the contrary, vagal cardiomotor tone is considered to be functionally unopposed because the vagus nerve remains anatomically intact (Inoue et al. 1995). In animal models of SCI, the abundance of Ca^{2+} regulatory proteins is altered in a manner that increases the susceptibility to ventricular arrhythmias by reducing the inducible threshold to programmed electrical stimulation by as much as 48% (Rodenbaugh et al. 2003a, b).

Thus, chronic deprivation and/or dysregulation of cardiac sympathovagal balance may be influential in altering expression and density of Ca^{2+} ion channel proteins, thereby potentially affecting nNOS expression, and lowering the arrhythmic inducible threshold. Pharmaceutical interventions focused on increasing arterial blood pressure that either directly or indirectly affect cardiac NO levels, in the presence of depressed sympathetic neuromodulation of the heart, may interfere with normal action potential kinetics and increase the propensity for atrioventricular conduction blockade or ventricular arrhythmia.

Two prospective studies were performed in persons with tetraplegia and demographically matched neurologically intact control subjects to determine the effects of acute systemic NOS inhibition, which induces hypertension, with several different weight-based doses of intravenous L-NAME, on electrocardiogram (ECG) indices of cardiac conduction. Our primary objective was to determine the effects of L-NAME during supine rest on PQ and QT interval durations in persons with tetraplegia who have cardiac sympathetic dysfunction, compared to neurologically intact control subjects. In addition, we determined whether a systemic hemodynamic provocation (i.e., head-up tilt test; HUT) known to induce sympathetic nervous system (SNS) responses might unmask differential effects of L-NAME on cardiac conductance in persons with tetraplegia.

2. Investigations, results and discussion

This was a two-part investigation that evaluated the effects of different weight-based doses of L-NAME in the supine and HUT position in two separate cohorts: persons with tetraplegia and age-matched, neurologically-intact control subjects. Seven subjects with tetraplegia and 6 age-matched control subjects completed Study 1; 7 subjects with tetraplegia and 7 age-matched control subjects completed Study 2. All subjects were between the ages of 22 and 54 years old with no known history of cardiovascular disease, pulmonary disease, or diabetes mellitus. To be considered eligible for study participation, subjects had to be current non-smokers for a minimum of one year prior to the investigation and not taking medications with known effects on cardiovascular autonomic function. The two research studies were approved by the Institutional Review Board of the James J. Peters Veterans Affairs Medical Center, and separate written informed consent was obtained from each subject prior to the initiation of the respective study procedures. Human use of L-NAME for these protocols was approved by the Food and Drug Administration under the investigational new drug number 67,134.

Demographic data for Study 1 and Study 2 cohorts are provided in Table 1. Seven subjects with tetraplegia and 6 age-matched control subjects participated in Study 1; 7 subjects with tetraplegia and 7 age-matched control subjects participated in Study 2. The cohorts for each study group were well matched for age, height, weight, and BMI. No significant differences were noted between or within groups at each visit at baseline for ECG variables (Table 2). No main or interaction effects were observed during Study 1 for the respective ECG intervals. From a clinical perspective, there were no instances of clinically relevant prolongation or shortening of the respective ECG intervals at baseline, or in response to the doses of L-NAME in either group. MAP increased after study drug administration in a dose-dependent manner for each group after infusion, an effect which was maintained for 1 additional hour post-infusion. Changes to MAP during L-NAME administration throughout the trial did not exceed *a priori* hemodynamic restrictions (Table 3).

No statistical differences were noted between the groups at the placebo visit for baseline ECG variables (Table 4). In addition, no significant ECG interval differences were observed at baseline across visits within the group with tetraplegia. For the placebo visit, the main effects for group and orthostatic position or interaction effect were not significant for HR or the ECG variables of interest (Table 4). Similarly, in the group with tetraplegia, there were no visit or interaction effects observed during Study 2 for the respective ECG intervals. There were no instances of clinically significant prolongation or shortening of the respective ECG intervals at baseline or in response to the intervention in either group. There were no significant group differences in baseline MAP. In subjects with tetraplegia, MAP increased from baseline to 60 min with L-NAME $1\,mg{\cdot}kg^{-1}$ ($p < 0.02$) and $2\,mg{\cdot}kg^{-1}$ ($p < 0.0001$), but declined with time after assumption of the HUT position with the lower dose, but MAP was maintained at significantly higher levels with HUT at the higher dose (Table 5).

Our report is the first description of the *in vivo* effects of NOS inhibition on cardiac conduction assessed by ECG intervals in humans that we are aware of in the literature. Intravenous L-NAME infusion, when administered at weight-based doses that do not induce hypertensive crises, is without acute consequential effects to cardiac dromotropism or other indices of cardiac conduction. In addition, the presence of cardiac autonomic dysfunction in the group with tetraplegia was not associated with any cardiac conductance disturbances after NOS inhibition, in contrast to pharmacologic blockade of the autonomic nervous system after L-NAME in an animal model (Fellet et al. 2006). Similarly, L-NAME administration during a postural change (i.e., HUT), which imparts a systemic challenge to the cardiovascular system, did not appear to affect cardiac conduction. It may be inferred that production of cardiac NO (or the presence of NOS in cardiac tissues) and its effect on cardiac conduction in persons with tetraplegia, is functionally similar to that of control subjects. It also appears that NOS inhibition has no effect on the electrophysiological effects of vagal cholinergic dysfunction in persons with SCI (Wecht et al. 2006, 2009b).

Table 1: Group demographics

	Study 1- Supine		Study 2- Orthostasis	
	Tetraplegia	Control	Tetraplegia	Control
	(n = 7)	(n = 6)	(n = 7)	(n = 7)
Age (y)	38 ± 10	32 ± 8	41 ± 9	33 ± 9
Height (m)	1.74 ± 0.06	1.78 ± 0.13	1.74 ± 0.07	1.73 ± 0.08
Weight (kg)	72.9 ± 15.1	76.4 ± 15.6	71.8 ± 11.0	70.4 ± 9.4
BMI (kg/m^2)	24.0 ± 4.1	24.1 ± 2.9	23.5 ± 1.5	23.4 ± 1.5
DOI (y)	18 ± 11	–	21 ± 12	–
Complete Injury (n)	2	–	1	–

BMI = body mass index; DOI = duration of injury. Data are expressed as group mean ± SD.

Table 2: Study 1-supine: outcomes of cardiac conduction and repolarization

	Group	Dose ($mg \cdot kg^{-1}$)	Baseline	60	120
HR (bpm)	Control	0	57 ± 9	60 ± 9	59 ± 10
	Tetraplegia	0	57 ± 12	58 ± 12	60 ± 13
	Control	1	59 ± 10	54 ± 9	54 ± 10
	Tetraplegia	1	58 ± 13	48 ± 10	49 ± 10
	Control	2	60 ± 10	48 ± 5	50 ± 7
	Tetraplegia	2	59 ± 9	45 ± 7	47 ± 7
	Control	4	64 ± 12	51 ± 5	51 ± 4
	Tetraplegia	4	66 ± 2	46 ± 5	46 ± 5
PQ (ms)	Control	0	129 ± 22	130 ± 4	132 ± 23
	Tetraplegia	0	120 ± 15	122 ± 18	121 ± 18
	Control	1	125 ± 18	129 ± 19	127 ± 17
	Tetraplegia	1	122 ± 20	126 ± 21	127 ± 25
	Control	2	127 ± 19	129 ± 18	122 ± 31
	Tetraplegia	2	123 ± 20	128 ± 23	127 ± 21
	Control	4	118 ± 19	118 ± 20	117 ± 18
	Tetraplegia	4	112 ± 8	119 ± 17	120 ± 20
QT (ms)	Control	0	374 ± 41	374 ± 44	371 ± 30
	Tetraplegia	0	366 ± 47	370 ± 49	363 ± 48
	Control	1	367 ± 41	384 ± 48	379 ± 42
	Tetraplegia	1	368 ± 45	384 ± 48	380 ± 49
	Control	2	357 ± 80	392 ± 48	385 ± 39
	Tetraplegia	2	349 ± 58	383 ± 41	377 ± 41
	Control	4	360 ± 24	376 ± 19	382 ± 18
	Tetraplegia	4	340 ± 23	364 ± 30	364 ± 32
QTC (ms)	Control	0	362 ± 29	322 ± 95	317 ± 103
	Tetraplegia	0	357 ± 21	357 ± 18	355 ± 22
	Control	1	362 ± 33	352 ± 33	360 ± 38
	Tetraplegia	1	358 ± 23	340 ± 19	341 ± 17
	Control	2	366 ± 31	349 ± 32	347 ± 26
	Tetraplegia	2	355 ± 20	329 ± 20	331 ± 14
	Control	4	371 ± 16	348 ± 2	349 ± 5
	Tetraplegia	4	358 ± 17	317 ± 11	334 ± 38

Data are expressed as group mean ± SD.

Table 3: Study 1-supine: mean arterial pressure

	Group	Dose ($mg \cdot kg^{-1}$)	Baseline	60	120 (1 Hour Post)
MAP (mmHg)	Control	0	84 ± 8*	91 ± 13	91 ± 14
	Tetraplegia	0	73 ± 11	78 ± 14	82 ± 14
	Control	1	88 ± 9*	81 ± 27	97 ± 14
	Tetraplegia	1	76 ± 8	96 ± 12¶	98 ± 12§
	Control	2	87 ± 9	103 ± 16‡	103 ± 17†
	Tetraplegia	2	78 ± 10	111 ± 10¶	107 ± 5‡
	Control	4	85 ± 8	100 ± 14	95 ± 5
	Tetraplegia	4	88 ± 7	112 ± 9‡	113 ± 7‡

Data are expressed as group mean ± SD. Group Differences: $^{*}p<0.05$.
Significant change from baseline: $^{†}p<0.05$; $^{‡}p<0.01$; $^{¶}p<0.001$; $^{§}p<0.001$.

Our findings differ from a prior animal model that demonstrated a paradoxical and simultaneous increase in HR and blood pressure to L-NAME administration during autonomic blockade, albeit at a dose concentration that exceeded our study design (i.e., $7.5\,mg \cdot kg^{-1}$) (Fellet et al. 2006). Persons with SCI are appreciated to have residual efferent cardiac autonomic modulation, which is not comparable to pharmacologic blockade of the autonomic pathways in an able-bodied cohort (Inoue et al. 1995; Grimm et al. 1997; Wang et al. 2000). The pressor effect from L-NAME precluded our ability evaluate the autonomic contribution to the observed HR changes. With this appreciation, the L-NAME doses used in our study were substantially lower than those employed in the animal study (i.e., 1.0 or $2.0\,mg \cdot kg^{-1}$ vs. $7.5\,mg \cdot kg^{-1}$); administration of higher doses than $4.0\,mg \cdot kg^{-1}$ may be difficult to safely achieve, as evidenced by attrition at our highest dose of agent.

The tetraplegia cohort used in this study was comprised of otherwise healthy individuals, who were medically stable and free of acute medical conditions. Because of these narrow selection criteria, it would be inappropriate to generalize our findings to other subject populations. It is possible that indices of cardiac conduction in persons with SCI who are predisposed to or have recurrent episodes of autonomic dysreflexia, or those who have underlying cardiac pathology, may respond differently to L-NAME infusion. IV administration of L-NAME has a well appreciated systemic impact. Thus, as a non-specific inhibitor

Table 4: Study 2-orthostasis: outcomes of cardiac conduction and repolarization

	Group	Dose ($mg \cdot kg^{-1}$)	Baaseline	60	Post-Tilt 1	Post-Tilt 2
HR (bpm)	Control	0	61 ± 7	62 ± 9	65 ± 6	67 ± 9
	Tetraplegia	0	66 ± 12	63 ± 10	66 ± 11	69 ± 8
	Tetraplegia	1	65 ± 14	56 ± 10	63 ± 14	68 ± 14
	Tetraplegia	2	63 ± 12	46 ± 7	57 ± 11	63 ± 13
PQ (ms)	Control	0	110 ± 17	110 ± 19	112 ± 18	113 ± 17
	Tetraplegia	0	110 ± 27	94 ± 44	108 ± 26	110 ± 27
	Tetraplegia	1	114 ± 29	124 ± 28	115 ± 28	113 ± 26
	Tetraplegia	2	117 ± 19	130 ± 24	124 ± 25	120 ± 30
QT (ms)	Control	0	363 ± 30	366 ± 37	354 ± 35	351 ± 36
	Tetraplegia	0	358 ± 36	366 ± 38	356 ± 41	361 ± 29
	Tetraplegia	1	364 ± 34	377 ± 40	362 ± 41	354 ± 40
	Tetraplegia	2	366 ± 38	394 ± 37	371 ± 40	357 ± 43
QTC (ms)	Control	0	364 ± 20	368 ± 17	371 ± 22	372 ± 21
	Tetraplegia	0	372 ± 17	372 ± 22	370 ± 28	386 ± 21
	Tetraplegia	1	373 ± 18	357 ± 12	364 ± 16	372 ± 16
	Tetraplegia	2	372 ± 17	341 ± 20	360 ± 13	367 ± 14

Data are expressed as group mean ± SD.

Table 5: Study 2-orthostasis: mean arterial pressure

	Group	Dose ($mg \cdot mg^{-1}$)	Baseline	60	Post-Tilt 1	Post-Tilt 2
MAP (mmHg)	Control	0	85 ± 11	90 ± 9	90 ± 8	78 ± 20
	Tetraplegia	0	81 ± 16	77 ± 13	76 ± 11	67 ± 12
	Tetraplegia	1	81 ± 11	107 ± 17*	93 ± 15	80 ± 7
	Tetraplegia	2	77 ± 10	116 ± 23†	114 ± 18‡	140 ± 27‡

Data are expressed as group mean ± SD. Significant change from baseline: $^{*}p<0.05$; $^{\dagger}p<0.0001$; and $^{\ddagger}p<0.001$.

of NOS activity, use of this drug to raise blood pressure has the potential to acutely and adversely affect bodily systems that require NO. Because of this and the paucity of large-scale or chronic administration studies conducted in neurologically comprised clinical cohorts describing the human experience of L-NAME, it would be premature to make inferences of the safety of L-NAME beyond its current acute experimental use.

Limitations: Although we had a well-matched cohort of individuals for our analyses, a larger and more carefully neurologically-defined subject sample is required to corroborate our findings and to more definitely exclude any possible relationship(s) between cardiac NO levels and vagal chronotropism in persons with SCI. In addition, the introduction of autonomic blockade in persons with SCI (and neurologically intact controls) during L-NAME administration, at comparable dose concentrations used by us in this report may provide additional insight as to its effect on cardiac electrophysiology. Our data acquisition system relied on a 3-lead configuration (Lead II), inclusion of a systematic 12-lead ECG may have provided more detailed information for evaluation. The analysis software employed a filtering algorithm to improve signal resolution and assist in the temporal identification of events; application of this methodology may have had an effect on the data; however it should be appreciated that the same filter was uniformly applied in all analyses.

Acute non-specific NOS inhibition by L-NAME administration at dose levels previously used in clinical investigation does not appear to impart adverse or statistically significant changes in ECG indices of cardiac conduction. These affects were observed in both the supine and orthostatic positions in persons with tetraplegia, and while supine in demographics-matched neurologically intact control subjects. This acute observation, although novel, requires further study and verification in a larger cohort of subjects with cardiac autonomic dysfunction. Similarly, chronic or repeated administration requires further study and evaluation because acute administration of L-NAME or NOS inhibitors may not provide sufficient time to evaluate alterations systemic effects from repeat therapeutic doses of the drug.

3. Experimental

3.1. Procedures

Subjects were instructed to be well hydrated on each study visit and to avoid heavy exertion, caffeine, and alcoholic beverages for a minimum of 24 h prior to testing. Data collections commenced between 8:00 and 10:00 AM and were completed in a dimly lit, temperature controlled room. Drug preparations were performed by the same research pharmacist on the morning of each test day. The equivalent volume of L-NAME was delivered in 100 ml of normal saline. Mean arterial pressure (MAP) is provided for descriptive purposes at the respective time points in each study. For a detailed explanation of the intervention and hemodynamic outcomes, the reader is directed to the primary reports of the parent investigations (Wecht et al. 2008, 2009a).

Study 1: Subjects entered the laboratory on as few as three, but up to 4 times to complete a non-randomized, open-label trial of intravenous administration of L-NAME (Clinalfa, Hauptstrasse 144, 4416 Bubendorf, Switzerland) at specific weight-based doses [i.e., 1 $mg \cdot kg^{-1}$, 2 $mg \cdot kg^{-1}$ or 4 $mg \cdot kg^{-1}$] or placebo infusion (normal saline) in the supine position. Progression to the next higher dose of agent was determined by a blood pressure response not to exceed a systolic pressure of 150 mmHg and/or diastolic pressure of 90 mmHg at the dose administered prior to dose escalation. Due to these hemodynamic restrictions, only 3 subjects from each group progressed to the 4 $mg \cdot kg^{-1}$ L-NAME dose. At each study visit subjects were transferred to the supine position in a standard hospital bed, instrumented (details to follow) and permitted a 20-minute acclimation period prior to baseline data collection. A catheter was inserted into the antecubital vein for intravenous administration of study drug, which was infused at a steady rate over 60 minutes with an automated pump (IVAC Signature Edition Gold, Model 7130E, Alaris Medical Systems, Carefusion, San Diego, CA). Data were collected at baseline prior to initiating L-NAME infusion (0 min), upon completion of infusion at 60 min (60 min), and 60 min post-infusion (120 min).

Study 2: Subjects with tetraplegia entered the laboratory on up to three occasions to complete a non-randomized, open-label trial of intravenous administration of placebo (normal saline) or L-NAME at 2 dose concentrations [i.e., 1 mg·kg^{-1}and 2 mg·kg^{-1}]. Because of the hemodynamic restrictions delineated in Study 1, only 6 of these subjects completed all 3 visits. For each visit, intravenous preparations and drug administration were identical to Study 1. Subjects were transferred and secured to a standard motorized tilt table, instrumented and permitted a 20-minute acclimation period prior to baseline data collection. For a detailed explanation of the tilt-table intervention and hemodynamic outcomes, please refer to the primary report of the parent investigation (Wecht et al. 2009a). After IV infusion of placebo or study drug, subjects underwent a progressive HUT maneuver from supine to 45°. Adjustment of the tilt table to each angle was accomplished in less than 5 seconds, and the progressive HUT maneuver consisted of 5 minutes at each intermediate angle of tilt (15°, 25° and 35°) and 30 minutes at 45°. For the purposes of this report, data are reported from baseline prior to infusion (0 min), upon completion of infusion at 60 min in the supine position (60 min), and at two 10 min intervals apart in the 45° HUT position (Post-tilt 1 and Post-tilt 2, respectively).

3.2. Instrumentation and data analysis

At each study visit, a continuous digital 3-lead ECG (Lead II) was collected for 5 min at the specified time points. All digital ECG data were collected at a sampling rate of 500 Hz with a 12-bit analog-to-digital converter (SCB 68, National Instruments, Austin, TX USA) using a customized program created with LabVIEW (National Instruments, Austin, TX USA). Data were archived on a computer hard drive for offline analysis. ECG site preparation was performed according to clinical standards (Drew et al. 2004) and placement of three electrodes was standardized (modified limb leads placed distal to the mid-clavicle bilaterally and precordial lead V5) for continuous heart rate monitoring (742 Mennen Medical ECG monitor, Bio-Medical Equipment Service, Louisville, KY). Two electrodes were placed on the trunk (sternum and proximal to the umbilicus) for monitoring of respiration rate via impedance pneumography (RESP 1, UFI, Morro Bay, CA). During data collection, subjects were instructed to breathe spontaneously.

Analysis of the digital ECG was performed on the longest duration of stable, ectopy and artifact free data from each time point, which was between 180 and 300 s in duration, using WinCPRS software (Absolute Aliens Oy, version 1.161, Turku, Finland). A first derivative detection routine was used, which computes the derivative from four consecutive data points to facilitate temporal identification of the P, Q, R and T waves. A peak detection algorithm identified all R waves for use in generating the RR interval, which was subsequently converted to heart rate (HR = 60,000/RR). The PQ interval was defined as the elapsed time from the onset of the P wave to the onset of the Q wave. The QT interval was calculated as the time elapsed from the onset of the Q wave to the terminal of the T wave (QT). To correct for heart rate dependency, the QT interval was corrected using the Bazett formula (Bazett 1920).

3.3. Statistical analysis

Values are expressed as group mean ± standard deviation (SD). Unpaired t-tests were performed to identify group (tetraplegia vs. control) differences among demographic variables (age, height, weight, and BMI) in each study cohort. In addition, unpaired t-tests were performed to identify group differences among baseline ECG variables (HR, PQ, QT, and QTC intervals) within each visit for both studies. Factorial analysis of variance (ANOVA) was used to identify baseline ECG differences across visits within each group for both studies. In Study 1, a 2 (group: tetraplegia, control) x 4 (visit: 0 mg·kg^{-1}, 1 mg·kg^{-1}, 2 mg·kg^{-1}, 4 mg·kg^{-1}) repeated measures ANOVA was performed to identify ECG responses to L-NAME. To further elucidate significant main effects for group, visit, or interaction effects, Scheffe's post-hoc paired t-tests were performed. In study 2, a 1 factor (group: tetraplegia, control) repeated measures ANOVA was performed to identify ECG differences between groups to 0 mg kg^{-1}. Furthermore in study 2 a 1 factor (visit: 0 mg·kg^{-1}, 1 mg·kg^{-1}, 2 mg·kg^{-1}) repeated measures ANOVA was performed to identify differences in the ECG responses to L-NAME dose during HUT in the group with tetraplegia. Separate repeated measures ANOVA were performed within each group for the respective L-NAME doses to describe MAP responses. An *a priori* level of significance was set at $p \leq 0.05$. Statistical analyses were completed using Statview 5.0 (SAS Institute, Inc.).

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