Published in final edited form as:

Auton Neurosci. 2012 September 25; 170(0): 66–69. doi:10.1016/j.autneu.2012.06.007.

***Bacillus anthracis* Lethal Toxin Induces Complex Changes in Sympathetic Nerve Discharge Regulation**

MJ Kenney, LJ Mosher, and RJ Fels

Department of Anatomy and Physiology, Kansas State University, Manhattan, KS

Abstract

Bacillus anthracis lethal toxin (LeTx) alters blood pressure and visceral sympathetic nerve discharge (SND) regulation (Garcia et al, 2012). The present results indicate that LeTx infusions produce similar response profiles in peripheral (lumbar) and visceral (renal) SND; an initial widespread activation of sympathetic nerve outflow, followed by a generalized reduction in lumbar and renal SND from peak levels, although the sympathoinhibition tended to be attenuated in lumbar SND. Combined hypoxia+hypercapnia during the hypotensive phase of LeTx infusions increased lumbar and renal SND, indicating that sympathetic neural circuits can be activated during the circulatory shock phase of *B. anthracis* septicemia.

Keywords

anthrax lethal toxin; LeTx; lumbar SND; arterial blood pressure

INTRODUCTION

Bacillus anthracis infection is a lethal pathophysiological condition associated with marked reductions in arterial blood pressure (Cui et al, 2004; Cui et al, 2005; Cui et al, 2006). The sympathetic nervous system (SNS) plays a critical role in cardiovascular regulation (Hosking et al, 2009; Kenney et al, 1998), however, a dearth of information is known regarding the effect of *B. anthracis* lethal toxin (LeTx) on regulation of sympathetic nerve discharge (SND). LeTx has been considered central to the pathogenesis of *B. anthracis* (Cui et al, 2004; Leepa, 2000; Mock et al, 2001). We recently determined the effects of LeTx infusions on visceral (renal, adrenal, splenic) SND regulation in baroreceptor-intact (INT) and sinoaortic-denervated (SAD) rats (Garcia et al, 2012). In both groups, LeTx infusions produced an initial state of cardiovascular and visceral SND activation, followed by progressive and marked reductions from peak levels in mean arterial pressure (MAP) and SND (renal, adrenal, splenic), indicating a lack of specificity in LeTx-induced visceral SND responses.

© 2012 Elsevier B.V. All rights reserved.

Correspondence to: Michael J. Kenney, PhD, Department of Anatomy and Physiology, Kansas State University, Coles Hall 228, Manhattan, KS 66506, Phone: 785-532-4513, Fax: 785-532-4557, Kenny@vet.k-state.edu.

DISCLOSURES

No conflicts of interest are declared by the authors.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The SNS is capable of generating complex outflow patterns including the generation of nonuniform nerve responses (Kanbar et al, 2008; Morrison, 2001). Whether the SND response profile to LeTx is similar in nerves innervating peripheral compared with visceral targets remains unanswered. The first goal was to determine the effect of continuous LeTx or vehicle infusions on the simultaneously recorded discharges in lumbar and renal sympathetic nerves in anesthetized INT and SAD rats. Experiments were completed in SAD rats to eliminate the influence of baroreceptor afferent feedback pathways on SND responses of central origin. Lumbar nerve recordings were completed because this nerve contains axons that innervate mainly the skeletal muscle and skin vasculatures of the hindlimb and tail (Baron et al, 1998; Kanbar et al, 2008) whereas renal SND recordings were considered a visceral nerve control.

LeTx-induced reductions in visceral SND, and possibly peripheral SND (depending on lumbar SND responses), may result from a generalized attenuation in the responsivity of sympathetic neural circuits. If this was the case then it would be expected that the responsivity of sympathetic neural circuits to other excitatory stressors, such as a short bout of combined hypoxia+hypercapnia (i.e., asphyxia) (Kenney, 1994; Kenney et al, 2002), would be markedly impaired during the final phase of LeTx infusions. On the other hand, if SND was markedly activated in response to asphyxia during LeTx infusions when SND was substantially reduced from peak levels, this would suggest that sympathetic neural circuits retain the capability to respond to acute stress. This would be an intriguing finding because SND activation, rather than inhibition, during the final phase of LeTx infusions would likely be an advantageous physiological response profile, at least from a blood pressure standpoint. Thus, the second goal was to determine the effect of combined hypoxia+hypercapnia applied during the final stages of LeTx infusions on the responsivity of lumbar and renal SND.

METHODS

Experiments were performed in accordance with the American Physiological Society's guiding principles for research involving animals and approved by the Institutional Animal Care and Use and Biosafety Committees.

General Procedures

Experiments were completed using male Sprague-Dawley rats (300–400 grams). Anesthesia was induced by isoflurane (3–5%), maintained during surgical procedures using isoflurane (1.25%–1.75%), α -chloralose (80 mg/kg ip), and urethane (800 mg/kg ip), and maintained during experiments using α -chloralose (35–45 mg/kg/hr iv) and urethane (200 mg/kg every 4 hours ip). The trachea was cannulated, rats were paralyzed with gallamine triethiodide (5–10 mg/kg iv, initial dose; 10–15 mg/kg/hr, maintenance dose), and artificially ventilated (Hosking et al, 2009). Femoral arterial pressure and colonic temperature were measured. In selected experiments sinoaortic denervations were completed 3–4 hours before initiation of experimental protocols (Hosking et al, 2009; Kenney, 1994). The adequacy of anesthesia was demonstrated by an inability of mechanical stimulation of the hindlimb to increase mean arterial pressure (MAP) and SND or elicit a withdrawal reflex.

Sympathetic Nerve Recordings

Sympathetic nerve recordings (lumbar and renal) were completed using methods described by Hosking et al (2009). Filtered neurograms were rectified and integrated (time constant 10 ms), quantified as microvolts $\times$ seconds ($\mu\text{V}\cdot\text{s}$), and corrected for background noise after ganglionic blockade or nerve crush.

Experimental Protocols

Rats received intravenous (femoral vein) LeTx [lethal factor (LF) and protective antigen (PA); LF 100 $\mu\text{g/kg}$ +PA 200 $\mu\text{g/kg}$; infusion rate 1.0 ml/hr) or vehicle (PA+PBS with 1% BSA, 0.5 ml/hr or PBS with 1% BSA, 1.0 ml/hr). LF and PA were recombinant proteins prepared from *B. anthracis* and purchased from List Biological Laboratories. Experiments were completed in INT and SAD rats. MAP, HR, lumbar SND, and renal SND were recorded continuously during LeTx or vehicle infusions. The maximum infusion duration was 3 hours; however, due to LeTx-induced reductions in MAP, the majority of experiments were terminated before the completion of 3 hours. The effect of a short bout of combined hypoxia and hypercapnia on SND responsivity, produced by shutting off the mechanical ventilator for a short period of time (15–30 s), was determined near the end of LeTx and vehicle infusion experiments. Arterial pO₂ and pCO₂ levels were measured before and 15–30 s after ventilation was terminated. Rats were euthanized by an intravenous overdose of methohexital sodium (150 mg/kg iv).

Statistical Analysis

Values are means $\pm$ SE. Control values of SND were considered as 100% and SND data are expressed as percentage change from control. Statistical analyses included Student *t*-tests and analysis of variance techniques with a repeated-measures design, followed by Bonferroni post hoc tests. $P < 0.05$ indicated statistical significance.

RESULTS

LeTx infusions were completed in 13 INT rats. Due to LeTx-induced reductions in MAP, 10 of the 13 experiments were terminated before completion of the 3-hour infusion protocol. These rats were identified as non-survivors and data (MAP and lumbar SND, $n=10$; renal SND, $n=9$) were analyzed as a group. LeTx infusions were completed in 10 SAD rats, 7 of which were classified as non-survivors (MAP and lumbar SND, $n=7$; renal SND, $n=6$). Data from the 6 survivors that completed the 3-hour LeTx infusion protocol were not included in the analysis. The average duration of LeTx infusion experiments was 115 ± 7 min in INT rats and 121 ± 10 min in SAD rats. Vehicle infusions were completed in 7 INT rats (MAP, $n=7$; renal SND, $n=6$; lumbar SND, $n=5$) and 6 SAD rats (MAP and renal SND, $n=6$; lumbar SND, $n=5$).

Figure 1 (LeTx, top panels) shows summarized data at four experimental points from LeTx infusion experiments in INT and SAD rats. 1) Control (C) prior to initiation of infusions; 2) the experimental point where peak (PK) increases in MAP and SND were observed during LeTx infusions; 3) the progressive decline (PD) phase in MAP from PK values, defined as the approximate mid-point between PK and nadir values; and 4) the nadir (ND) level of MAP, defined as the experimental point at which LeTx-induced reductions in MAP were at or near 50 mmHg. The ND level of MAP was considered as the final experimental point for SND. Figure 1 (Veh, bottom panels) shows summarized data from vehicle infusion experiments in INT and SAD rats before (C) and at 60, 120, and 180 min during the infusions.

The initial cardiovascular effect of LeTx was to increase MAP in SAD rats, however, as the LeTx infusion progressed, MAP was significantly reduced from PK values during the PD and ND phases in INT and SAD rats (Figure 1, LeTx, MAP). Initially, lumbar SND responses to LeTx infusions were characterized by significant increases from control values in INT and SAD rats (Figure 1, LeTx, Lumbar SND, PK). Subsequent to PK responses, ND levels of lumbar SND were reduced significantly from PK values in SAD but not INT rats (Figure 1, LeTx, Lumbar SND, ND). As expected, PK levels of renal SND were

significantly increased from control values, while renal SND was significantly reduced from PK levels during the PD and ND phases of LeTx infusions (Figure 1, LeTx, Renal SND). MAP and SND (lumbar and renal) were not reduced from control levels during vehicle infusions in INT or SAD rats (Figure 1, Veh).

Acute asphyxia at the ND level of MAP during LeTx infusions, and at the final experimental point during vehicle infusions, significantly increased lumbar and renal SND in INT (A) and SAD (B) rats (Figure 2). Acute asphyxia during LeTx and vehicle infusions produced hypoxia (arterial pO₂ levels: INT, 26.5 ± 2 mmHg; SAD, 28.1 ± 1 mmHg) and hypercapnia (arterial pCO₂ levels: INT, 60.2 ± 3 mmHg; SAD, 60.10 ± 1 mmHg).

DISCUSSION

The present results demonstrate that continuous LeTx infusions markedly influence SND regulation by producing an initial widespread activation of sympathetic nerve outflow (peripheral and visceral), followed by a generalized reduction in the level of SND directed towards the same targets, although, with regard to the sympathoinhibitory responses, lumbar and renal SND paralleled one another in a qualitative but not in a strict quantitative sense. Specifically, the progressive LeTx-induced hypotension was associated with significant reductions in renal SND from peak levels in INT and SAD rats, whereas lumbar SND was significantly reduced from peak levels in only SAD rats, although it tended to be decreased from peak levels in INT rats. Thus, during LeTx infusions both peripheral and visceral SND failed to demonstrate a continued state of activation and were characterized by significant reductions from peak levels, although the magnitude of the lumbar sympathoinhibition was attenuated compared with renal SND.

The lack of a marked reduction in lumbar SND during the hypotensive phase of LeTx infusions in INT rats may be due in part to unloading of the arterial baroreceptors, therefore we speculated that LeTx infusions in SAD rats would produce substantial reductions from peak levels in lumbar SND and elicit a more marked renal sympathoinhibitory response. Although lumbar SND was significantly reduced from peak levels during the hypotensive phase of LeTx infusions in SAD rats, the magnitude of change was not substantially different from INT rats. Moreover, renal SND was reduced to similar levels in SAD and INT rats. These data indicate that SND responses to LeTx were not markedly influenced by unloading of the arterial baroreceptors, suggesting that the central regulation of SND is altered during LeTx infusions.

The failure to activate SND during the hypotensive phase of LeTx infusions, coupled with the reduction of SND from peak levels during the hypotensive phase of LeTx infusions, suggested that LeTx may produce a generalized attenuation in the responsivity of sympathetic neural circuits. However, application of a short bout of asphyxia at ND levels of MAP markedly increased lumbar and renal SND, indicating that the SNS retained the capacity to produce excitation, yet failed to substantially activate peripheral and visceral SND during the PD and ND phases of LeTx infusions. This is an interesting physiological response profile because increased activation of SND during the hypotensive phase of LeTx infusions would likely be a beneficial physiological response. Collectively these data support the hypothesis that SND regulation is altered in a complex manner during LeTx infusions.

Although the anesthetic regimen employed is used often in studies designed to investigate autonomic regulation, anesthesia can affect SND regulation (Ramchandra et al, 2006; Shimokawa et al, 1998). However, determining if LeTx produces nonuniform SND regulation requires the simultaneous recording of discharges in two or more sympathetic

nerves, an experimental methodology that, at least in our hands, is best completed in anesthetized preparations. Studies involving SND recordings in human subjects often include direct recordings of muscle SND from the peroneal nerve (Wallin et al, 2007), consistent with the recording of lumbar SND in the rat. Therefore, lumbar SND recordings provide some degree of translational insight.

In summary, continuous LeTx infusions, used to model *B. anthracis* infection, markedly influenced SND regulation by producing an initial progressive activation of sympathetic nerve outflow (lumbar and renal), followed by a generalized reduction of SND from peak levels directed towards peripheral and visceral peripheral targets. The SNS retained the capacity to produce sympathoexcitation during the hypotensive phase of LeTx infusion, yet did not do so in the current experiments, further demonstrating the complex effect that LeTx infusions exert on SND regulation. Understanding the effects of LeTx on SND regulation may facilitate the elaboration of mechanisms mediating circulatory shock during *B. anthracis* septicemia.

Acknowledgments

Supported by National Heart, Lung and Blood Institute Grants HL-091342 and HL-092392.

References

1. Baron R, Jänig W, Kollman W. Sympathetic and afferent somata projecting in hindlimb nerves and the anatomical organization of the lumbar sympathetic nervous system of the rat. *J Comp Neurol*. 1998; 275:460–468. [PubMed: 3225349]
2. Cui X, Li Y, Li X, Haley M, Moayeri M, Fitz Y, Leppla SH, Eichacker PQ. Sublethal doses of *Bacillus anthracis* lethal toxin inhibit inflammation with lipopolysaccharide and *Escherichia coli* challenge but have opposite effects on survival. *J Infect Dis*. 2006; 193:829–840. [PubMed: 16479518]
3. Cui X, Li Y, Moayeri M, Choi GH, Subramanian GM, Li X, Haley M, Fitz Y, Feng J, Banks SM, Leppla SH, Eichacker PQ. Late treatment with a protective antigen-directed monoclonal antibody improves hemodynamic function and survival in a lethal toxin-infused rat model of anthrax sepsis. *J Infect Dis*. 2005; 191:422–434. [PubMed: 15633102]
4. Cui X, Moayeri M, Li Y, Li X, Haley M, Fitz Y, Correa-Araujo R, Banks SM, Leppla SH, Eichacker PQ. Lethality during continuous anthrax lethal toxin infusion is associated with circulatory shock but not inflammatory cytokine or nitric oxide release in rats. *Am J Physiol Regul Integr Comp Physiol*. 2004; 286:R699–709. [PubMed: 14715494]
5. Garcia AA, Fels RJ, Mosher LJ, Kenney MJ. *Bacillus anthracis* lethal toxin alters regulation of visceral sympathetic nerve discharge. *J Appl Physiol*. 2012; 112:1033–1040. [PubMed: 22114180]
6. Hosking KG, Fels RJ, Kenney MJ. Inhibition of RVLM synaptic activation at peak hyperthermia reduces visceral sympathetic nerve discharge. *Auton Neurosci*. 2009; 150:104–110. [PubMed: 19589733]
7. Kanbar R, Chapuis B, Oréa V, Barrés C, Julien C. Baroreceptor control of lumbar and renal sympathetic nerve activity in conscious rats. *Am J Physiol Regul Integr Comp Physiol*. 2008; 295:R8–R14. [PubMed: 18448616]
8. Kenney MJ. Frequency characteristics of sympathetic nerve discharge in anesthetized rats. *Am J Physiol*. 1994; 267:R830–840. [PubMed: 8092329]
9. Kenney MJ, Claassen DE, Bishop MR, Fels RJ. Regulation of the sympathetic nerve discharge bursting pattern during heat stress. *Am J Physiol*. 1998; 275:R1992–2001. [PubMed: 9843889]
10. Kenney MJ, Fels RJ. Sympathetic nerve regulation to heating is altered in senescent rats. *Am J Physiol Regul Integr Comp Physiol*. 2002; 283:R513–R520. [PubMed: 12121865]
11. Leppla, SH. Anthrax toxin. In: Aktories, KJ., editor. *Bacterial Protein Toxins*. New York: Springer; 2000. p. 445–472.
12. Mock M, Fouet A. Anthrax. *Annu Rev Microbiol*. 2001; 55:647–671. [PubMed: 11544370]

13. Morrison SF. Differential control of sympathetic outflow. *Am J Physiol Regul Integr Comp Physiol.* 2001; 281:R683–98. [PubMed: 11506981]
14. Ramchandra R, Barrett CJ, Guild SJ, Malpas SC. Evidence of differential control of renal and lumbar sympathetic nerve activity in conscious rabbits. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290:R701–R708. [PubMed: 16239371]
15. Shimokawa A, Kunitake T, Takasaki M, Kannan H. Differential effects of anesthetics on sympathetic nerve activity and arterial baroreceptor reflex in chronically instrumented rats. *J Auton Nerv Syst.* 1998; 72:46–54. [PubMed: 9760079]
16. Wallin BG, Charkoudian N. Sympathetic neural control of integrated cardiovascular function: insights from measurement of human sympathetic nerve activity. *Muscle Nerve.* 2007; 36:595–614. [PubMed: 17623856]

Figure 1.

MAP, lumbar SND, and renal SND responses to anthrax lethal toxin (**LeTx**, top panels) and vehicle (**Veh**, bottom panels) infusions in Intact (INT, open bars) and sinoaortic-denervated (SAD, closed bars) rats. LeTx data are reported at Control (C), Peak (PK), Progressive decline (PD), and Nadir (ND) points, see Results for details. *PK significantly different from condition-matched C. ‡PD and ND significantly different from condition-matched PK. †SAD significantly different from INT. Veh data are reported during control (C) and 60, 120, and 180 min after initiation of vehicle infusions.

Figure 2.

Lumbar and renal SND responses to a bout of combined hypoxia+hypercapnia (asphyxia) during the final minutes of vehicle (open bars) and LeTx (closed bars) infusions in Intact (INT, A) and sinoaortic-denervated (SAD, B) rats. SND levels are percent change from levels recorded prior to application of asphyxia. *Levels of SND during asphyxia significantly different from levels prior to asphyxia.