**Bacillus anthracis** lethal toxin alters regulation of visceral sympathetic nerve discharge

A. A. Garcia, R. J. Fels, L. J. Mosher, and M. J. Kenney

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

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Garcia AA, Fels RJ, Mosher LJ, Kenney MJ. *Bacillus anthracis* lethal toxin alters regulation of visceral sympathetic nerve discharge. *J Appl Physiol* 112: 1033–1040, 2012. First published November 23, 2011; doi:10.1152/japplphysiol.01105.2011.—*Bacillus anthracis* infection is a pathological condition that is complicated by progressive decreases in mean arterial pressure (MAP). Lethal toxin (LeTx) is central to the pathogenesis of *B. anthracis* infection, and the sympathetic nervous system plays a critical role in physiological regulation of acute stressors. However, the effect of LeTx on sympathetic nerve discharge (SND), a critical link between central sympathetic neural circuits and MAP regulation, remains unknown. We determined visceral (renal, splenic, and adrenal) SND responses to continuous infusion of LeTx [lethal factor (100 μg/kg) + protective antigen (200 μg/kg)] infused at 0.5 ml/h for ≤6 h] and vehicle (infused at 0.5 ml/h) in anesthetized, baroreceptor-intact and baroreceptor (sinoaortic)-denervated (SAD) Sprague-Dawley rats. LeTx infusions produced an initial state of cardiovascular and sympathetic nervous system activation in intact and SAD rats. Subsequent to peak LeTx-induced increases in arterial blood pressure, intact rats demonstrated a marked hypotension that was accompanied by significant reductions in SND (renal and splenic) and heart rate (HR) from peak levels. After peak LeTx-induced pressor and sympathoexcitatory responses in SAD rats, MAP, SND (renal, splenic, and adrenal), and HR were progressively and significantly reduced, supporting the hypothesis that LeTx alters the central regulation of sympathetic nerve outflow. These findings demonstrate that the regulation of visceral SND is altered in a complex manner during continuous anthrax LeTx infusions and suggest that sympathetic nervous system dysregulation may contribute to the marked hypotension accompanying *B. anthracis* infection.

**INHALATIONAL Bacillus anthracis infection** is a lethal pathophysiological condition and a major bioterrorism health threat (1, 9). *B. anthracis* produces two exotoxins: lethal toxin (LeTx) and edema toxin (17). LeTx consists of two components, protective antigen (PA), required for the receptor-specific uptake of the toxin by host cells, and lethal factor (LF), the enzymatic moiety of LeTx; edema toxin is composed of PA and edema factor (4, 6, 17). Patients affected by the 2001 outbreak of *B. anthracis* in the United States suffered from shocklike symptoms (1, 20, 22). After this outbreak, investigators at the National Institutes of Health (3–6, 26) developed a rodent model to determine mechanisms mediating cardiovascular alterations associated with *B. anthracis* infection. The initial studies focused on the pathophysiological consequences of LeTx (3–6), as this toxin has been considered central to the pathogenesis of *B. anthracis* (17, 23). Cui et al. (5, 6) used a dose of LeTx that allowed for physiological comparisons between survivors and nonsurvivors. They (5, 6) found that continuous LeTx infusions produced hypotension and bradycardia in nonsurvivors, but not in survivors, and reported that nonsurvivors demonstrated a substantial amount of variability in the time to lethality following initiation of LeTx infusion. Decreases in arterial blood pressure in response to continuous LeTx infusions were not associated with hypoxemia, substantial increases in serum cytokine or nitric oxide levels, or evidence of myocardial injury (6). These findings suggest that LeTx produces hypotension independent of effects on respiratory function and via mechanisms other than enhanced inflammatory cytokines and nitric oxide release.

The sympathetic nervous system plays a critical role in physiological regulation under basal conditions and in response to acute and chronic stressors. Sympathetic nerves innervating the heart, blood vessels, and visceral organs are tonically active, and central sympathetic neural circuits regulate processes essential for maintaining cardiovascular homeostasis by altering the level of sympathetic nerve outflow (11, 13–15, 18). The importance of sympathetic nerve discharge (SND) in cardiovascular regulation in rats is demonstrated by the immediate and marked reduction in arterial blood pressure produced by pharmacological blockade of autonomic ganglionic transmission (14). Although neurological complications are a fundamental component of *B. anthracis* infections (10, 12, 19, 21), little to nothing is known regarding the effect of *B. anthracis* infection on directly recorded SND, a critical link between central sympathetic neural circuits and regulation of arterial blood pressure.

Because direct SND recordings provide an output measure of central neural-generated sympathetic nerve outflow, in the present study we determined renal, splenic, and adrenal SND responses to continuous LeTx or vehicle infusions (maximum infusion duration was 6 h) in chloralose-urethane-anesthetized, baroreceptor-intact and -denervated Sprague-Dawley rats. Renal SND was recorded, because the sympathetic neural innervation to the kidney affects physiological responses involved in blood pressure regulation, including renal blood flow, renin release, and salt and water retention by the renal tubules (8, 16). The sympathetic innervation to the spleen influences the contractile state of the vasculature of the spleen, a visceral organ that receives a significant amount of cardiac output (7). Adrenal SND was recorded because of its involvement in the release of epinephrine from the adrenal medulla (28).

We speculated that continuous infusions of LeTx would influence SND regulation in at least one, if not several, possible ways. Because increasing the level of activity in sympathetic nerves is a critical mechanism by which mammals regulate physiological responses to acute stress, our initial hypothesis was that the onset and early phase of LeTx infusions would produce visceral sympathoexcitation and increased arterial...
blood pressure. Subsequently, we expected that arterial blood pressure would be reduced as the duration of the LeTx infusions was increased, leading us to consider the possibility of two different SND response profiles associated with LeTx-induced hypotension in baroreceptor-nerververed rats. On the basis of the fundamental relationship between baroreflex-mediated regulation of arterial blood pressure and visceral SND, one plausible hypothesis was that LeTx-induced reductions in arterial blood pressure might elicit marked baroreflex-mediated increases in visceral sympathetic nerve outflow. In this case, we would expect that the reflex-induced activation of visceral SND during LeTx-induced reductions in arterial blood pressure would be additive to the sympathoexcitatory observed during the initial phase of LeTx infusions, a physiological response designed to attenuate reductions in arterial blood pressure mediated via LeTx. An alternative hypothesis, based on the prominent role of SND in blood pressure regulation (14), is that LeTx reduces visceral SND from peak levels during a later phase of continuous infusions, an effect that may occur in parallel with LeTx-induced reductions in arterial blood pressure. This response profile would support the idea that inhibition of visceral SND contributes to LeTx-induced reductions in arterial blood pressure. Finally, to eliminate the influence of the baroreceptor-afferent feedback mechanisms that can alter SND responses of central origin, LeTx infusions were also completed in sinoaortic-denervated (SAD) rats. We speculated that if mean arterial pressure (MAP) and SND were reduced from peak levels during the final phase of continuous LeTx infusions in baroreceptor-intact rats, then LeTx infusions in SAD rats would substantially reduce visceral SND, supporting the hypothesis that the central regulation of sympathetic nerve outflow is altered by LeTx.

**METHODS**

The experimental procedures and protocols were performed in accordance with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” and approved by the Institutional Animal Care and Use and Biosafety Committees at Kansas State University.

*General procedures.* Experiments were completed using male Sprague-Dawley rats (300–400 g). Anesthesia was induced by isoflurane (3–5%) and maintained during surgical procedures using isoflurane (1.25–1.75%), α-chloralose (80 mg/kg ip), and urethane (800 mg/kg ip). Two catheters [polyethylene (PE)-10 and PE-50] were placed in the femoral vein. Maintenance doses of α-chloralose (35–45 mg kg-1·h-1) were administered intravenously, whereas maintenance doses of urethane (200 mg/kg every 4 h) were administered intraperitoneally. The adequacy of anesthesia during the initial surgical procedures was indicated by the absence of a withdrawal reflex in response to mechanical stimulation of the tail or hindlimb. The adequacy of anesthesia after the establishment of SND recordings and following initiation of neuromuscular blockade was indicated by an inability of mechanical stimulation of the hindlimb or tail to increase SND or MAP.

The trachea was cannulated with a PE-240 catheter, and rats were paralyzed with gallamine triethiodide (5–10 mg/kg iv initial dose, 10–15 mg kg-1·h-1 maintenance dose) and artificially ventilated (11) using a small animal ventilator. End-tidal CO2 was measured using a microcapnometer and maintained near 4.0% during experiments by adjustment of the frequency of ventilation. Femoral arterial pressure was monitored using a pressure transducer connected to a blood pressure analyzer. Heart rate (HR) was derived from the pulsatile arterial pressure output of the blood pressure analyzer. Core temperature was measured with a thermistor probe inserted ∼3–4 cm into the colon and kept at 37.5–37.8°C during surgical interventions by a temperature-controlled table.

To eliminate the influence of baroreceptor afferent feedback mechanisms that can alter SND responses of central origin, many of the experiments included in this study were completed in SAD rats. Bilateral denervation of the aortic arch was completed by section of the superior laryngeal nerve near its junction with the vagus nerve and removal of the superior cervical ganglion. Bilateral carotid sinus denervation was completed by removal of the adventitia from the area of the carotid sinus bifurcation. SADs were completed 3–4 h before initiation of experimental protocols. The coherence function relating arterial pressure to SND was used to demonstrate the efficacy of the denervation procedure (13). Coherence analysis provides a measure of the strength of linear correlation of two signals as a function of frequency. The lack of coherence between arterial pressure and SND at the frequency of the HR demonstrated a complete SAD (13).

*Sympathetic nerve recordings.* Activity was recorded biphaseically with a platinum bipolar electrode after capacity-coupled preamplification (30- to 3,000-Hz band pass) from renal, splenic, and adrenal sympathetic nerves. Sympathetic nerves were isolated retroperitoneally, and nerve-electrode preparations were covered with silicone gel to prevent exposure to room air. Filtered neurograms were routed to an oscilloscope and a nerve traffic analyzer, where sympathetic nerve potentials were full-wave rectified and integrated (10-ms time constant), which produced a smooth tracing of the synchronized discharges. Total power in renal, splenic, and adrenal SND was quantified as microvolts × seconds (μV·s) (11). SND recordings were corrected for background noise after ganglionic (renal and splenic) blockade with chlorisondamine (5 mg/kg iv) or nerve crush (adrenal).

*Experimental protocols.* After completion of the surgical procedures, anesthetized rats were allowed to stabilize for 60 min before initiation of experimental protocols. As described by Cui et al. (4, 6), rats received LeTx [LF (100 μg/kg) + PA (200 μg/kg)] infused at 0.5 ml/h or vehicle (PA + PBS with 1% BSA or PBS with 1% BSA infused at 0.5 ml/h). LF and PA are recombinant proteins prepared from *B. anthuracis* and purchased from List Biological Laboratories. Experiments were completed in intact and SAD rats. LeTx or vehicle was infused intravenously via a femoral venous catheter, and the dose of LeTx was chosen on the basis of the time course of physiological responses to intravenous LeTx infusions as reported by Cui and colleagues (6). MAP, HR, and SND were recorded continuously during intravenous LeTx or vehicle infusions. The maximum infusion duration was 6 h; however, because of LeTx-induced reductions in arterial blood pressure, the majority of experiments were terminated before 6 h. Additional experiments involved the intravenous infusion of sodium nitroprusside (SNP) at a dose (20 μg/kg) that reduced basal levels of MAP to 50–60 mmHg. At the end of experiments, rats were euthanized by an overdose of methohexital sodium (150 mg/kg iv).

*Data collection and statistical analysis.* A computer-based ADInstruments Powerlab data acquisition system was used to collect all experimental data. Values are means ± SE. Control values of SND were considered as 100%, and renal, splenic, and adrenal SND data are expressed as percent change from baseline. Statistical analyses included Student’s *t*-tests and analysis of variance techniques with a repeated-measures design, followed by Bonferroni’s post hoc tests. The overall level of statistical significance was *P* < 0.05.

**RESULTS**

LeTx infusions were completed in nine rats with intact baroreceptors. Because of LeTx-induced reductions in MAP, eight of nine experiments were terminated before completion of the 6-h infusion protocol. These rats were classified as nonsurvivors, and data (MAP, *n* = 8; HR, *n* = 8; renal SND, *n* = 6; splenic SND, *n* = 6; and adrenal SND, *n* = 8) from these experiments were analyzed as a group. LeTx infusions...
were completed in nine SAD rats, and eight experiments were terminated before completion of the 6-h infusion protocol because of LeTx-induced hypotension. Data (MAP, n = 8; HR, n = 8; renal SND, n = 6; splenic SND, n = 6; and adrenal SND, n = 8) from the nonsurviving SAD rats were analyzed as a group. Results from the two rats that completed the 6-h LeTx infusion protocol were not included in the data analysis. Vehicle infusions were completed in nine rats with intact baroreceptors (MAP, n = 9; HR, n = 9; renal SND, n = 6; splenic SND, n = 7; and adrenal SND, n = 5) and in 9 SAD rats (MAP, n = 9; HR, n = 9; renal SND, n = 8; splenic SND, n = 5; and adrenal SND, n = 2).

Cardiovascular and SND responses from representative LeTx and vehicle (Veh) infusion experiments are shown in Figs. 1 and 2. Infusions in these experiments were maintained for 200 min; data are presented at 15-min intervals for the first 180 min and at 5-min intervals during the final 20 min of the infusion protocols. MAP, HR, and renal, splenic, and adrenal SND were progressively increased from control levels during the first several hours of LeTx infusion in the intact (Fig. 1) and SAD (Fig. 2) rat. In the intact rats (Fig. 1), peak MAP and HR responses occurred 180 min after initiation of LeTx infusion, whereas peak SND responses were evident 185 min after initiation of LeTx infusion. Subsequently, cardiovascular and SND parameters were reduced from peak values during the final minutes of LeTx infusion, with the most prominent decreases in SND occurring along with decreases in MAP. In the SAD rats (Fig. 2), peak MAP and HR responses were evident 150 min after initiation of LeTx infusion and peak SND responses occurred 165 min after initiation of LeTx infusion. Moreover, the recorded parameters were substantially reduced from peak values during the final 20–25 min of LeTx infusion. In contrast, MAP, HR, and SND did not substantially change from control levels during vehicle infusions in the intact (Fig. 1) or SAD (Fig. 2) rats.

Summarized cardiovascular and SND data from continuous LeTx infusions in intact and SAD rats are presented at four experimental points in Fig. 3: 1) control (C), before initiation of LeTx infusions; 2) the point at which peak (PK) LeTx-induced increases were observed; 3) the progressive decline (PD) in cardiovascular and SND levels from peak values, identified as the approximate midpoint between PK and nadir values; and 4) the point where MAP reached nadir (ND), identified primarily as a level of MAP at or near 50 mmHg. This level of MAP was chosen as the final experimental point, because further reductions in MAP would likely compromise brain blood flow and influence central regulation of SND, thereby confounding potential effects of LeTx on central sympathetic neural circuits. Levels of HR and SND recorded at the ND level of MAP were considered the final experimental point for these variables.

The initial responses to continuous LeTx infusions were characterized by significant increases from control levels in MAP, HR, and renal and adrenal SND in intact rats and MAP, HR, and renal, splenic, and adrenal SND in SAD rats (Fig. 3). Subsequently, in intact rats, MAP and renal SND were significantly reduced from PK values during the PD phase (Fig. 3), and ND levels of MAP, HR, and renal and splenic SND were significantly reduced from PK values (Fig. 3). Subsequent to PK responses in SAD rats, levels of MAP, HR, and renal SND recorded during the PD phase were significantly reduced from PK values (Fig. 3), whereas the ND levels of MAP, HR, and renal, splenic, and adrenal SND were significantly reduced...
from PK values (Fig. 3). PK HR and splenic SND responses to LeTx were significantly higher in SAD than intact rats.

In separate experiments using the same anesthetic protocol used for LeTx and vehicle infusion experiments, SND responses to SNP-induced reductions in MAP were determined in intact \((n=4)\) and SAD \((n=5)\) rats. Intravenous SNP significantly reduced MAP in intact \((-44 \pm 4\ mmHg)\) and SAD \((-63 \pm 5\ mmHg)\) rats, whereas reflex SND activation to
reduced MAP was evident in intact (renal SND, +121 ± 18%; splenic SND, +83 ± 19%; and adrenal SND, +86 ± 18%), but not SAD (renal SND, +3 ± 1%; splenic SND, +2 ± 2%; adrenal SND, +6 ± 3%), rats.

The duration of individual LeTx infusion experiments varied with the time at which the ND level for MAP was recorded. Specifically, the duration of LeTx infusion experiments in intact rats ranged from 127 to 255 min, with an average duration of 192 ± 18 min, whereas the duration of experiments in SAD rats ranged from 150 to 345 min, with an average duration of 214 ± 20 min. To determine the temporal relationships between cardiovascular and SND variables during LeTx infusions, experimental times were normalized by setting the ND point for each LeTx infusion to 100% and calculating relative PK and PD experimental times for MAP, HR, and SND. Normalized data are summarized in Fig. 4. Relative experimental times for PK increases in MAP and HR during LeTx infusions in intact rats occurred significantly before PK LeTx-induced adrenal SND responses and tended to precede PK renal and splenic responses, although these latter responses were not significantly different. Similarly, relative experimental times for PK increases in MAP and HR during LeTx infusions in SAD rats tended to, but did not significantly, precede PK SND (renal, splenic, and adrenal) responses. Normalized experimental times for PK adrenal SND responses differed significantly between intact and SAD rats. Relative experimental times for the PD point did not differ between MAP, HR, and SND (renal, splenic, adrenal) in intact or SAD rats.

Experimental times identifying average LeTx-induced changes in MAP were used as a temporal template for establishing appropriate time points from vehicle control experiments for comparison with LeTx infusion experiments. During continuous LeTx infusions in intact rats, PK increases in MAP occurred at 153 ± 14 min, ND levels of MAP occurred at 192 ± 18 min, and the longest duration of an individual LeTx infusion experiment was 255 min. During LeTx infusions in SAD rats, PK increases in MAP occurred at 141 ± 20 min, ND levels of MAP occurred at 214 ± 20 min, and the longest duration of an individual LeTx infusion experiment was 345 min. On the basis of these temporal data, cardiovascular and SND results from continuous vehicle infusion experiments are presented before (C) and at 2.5, 3, and 4.25 h during vehicle infusions in intact rats and before (C) and at 2.5, 3.5, and 5.75 h during vehicle infusions in SAD rats (Fig. 5). For ease of presentation, the final vehicle infusion points included in Fig. 5 are grouped.
in a time range identified as 4–6 h (rather than 4.25–5.75 h). MAP, HR, and SND (renal, splenic, and adrenal) were not reduced from control levels during continuous vehicle infusions in intact or SAD rats, and in contrast to LeTx experiments, HR and SND tended to increase from control levels as the duration of vehicle infusions was extended.

DISCUSSION

The present study is the first to determine the effects of LeTx infusion on visceral SND, an important link between central sympathetic neural circuits and physiological regulation. The present study revealed three new findings. 1) LeTx infusions in rats with intact arterial baroreceptors and in SAD rats produced an initial state of cardiovascular and sympathetic nervous system activation, as demonstrated by LeTx-induced increases in MAP, HR, and visceral SND. 2) Subsequent to peak LeTx-induced pressor and sympathoexcitatory responses, MAP in intact rats was characterized by a progressive and marked hypotension. On the basis of the fundamental relationship between reductions in arterial blood pressure and baroreflex-mediated activation of sympathetic nerve outflow, it might be expected that LeTx-induced decreases in MAP would elicit prominent activation of visceral SND. However, progressive reductions in MAP during LeTx infusions in intact rats were at no time associated with reflex-induced sympathoexcitation or tachycardia; in fact, SND (renal and splenic) and HR were significantly reduced from peak levels as LeTx infusions progressed, responses that paralleled the progressive hypotension. In contrast, SND was significantly increased in response to reductions in MAP produced by SNP, demonstrating that unloading of the arterial baroreceptors increases SND in urethane-chloralose-anesthetized, baroreceptor-innervated rats that have not been pretreated with LeTx. 3) Subsequent to peak LeTx-induced pressor and sympathoexcitatory responses in SAD rats, MAP, HR, and SND (renal, splenic, and adrenal) were progressively and significantly reduced. Because SAD eliminates baroreceptor afferent feedback mechanisms that can influence SND responses of central origin, these findings in SAD rats support the hypothesis that the central regulation of sympathetic nerve outflow is modulated during continuous LeTx infusions. Collectively, these data demonstrate that the regulation of visceral SND is altered in a complex manner during continuous anthrax LeTx infusions.

It is well established that the autonomic nervous system, with a particular focus on the sympathetic nervous system, plays a critical role in the acute regulation of arterial blood pressure. Kenney et al. (14) reported that pharmacological blockade of ganglionic transmission under basal conditions in anesthetized rats markedly reduces MAP and eliminates efferent SND, demonstrating that the sympathetic nervous system is tonically active and contributes to the basal regulation of arterial blood pressure. In addition, ganglionic blockade during acute increases in internal body temperature eliminates heat-induced elevations in MAP and SND (14), demonstrating that activation of the sympathetic nervous system is a prominent effector in arterial blood pressure regulation during periods of acute physical stress. What role might changes in regulation of visceral SND play in mediating the marked hypotension during a later phase of LeTx infusions? The present results indicate that, with the exception of adrenal SND responses to LeTx in intact rats, the relative experimental times for peak increases in SND and MAP during LeTx infusions did not differ statistically. However, it must be noted that peak LeTx-induced increases in SND tended to occur after peak increases in MAP, suggesting that inhibition of SND was likely not the initiator of the hypotensive response to LeTx. More-
over, although ND levels of SND were, for the most part, significantly reduced from peak levels, a substantial amount of visceral SND remained (compared with baseline levels) when MAP had reached its ND level, suggesting that factors other than reductions in visceral SND played a role in mediating the substantial hypotension to LeTx infusion. However, on the basis of the observations that SND did not continue to increase when MAP began to decrease during LeTx infusions in intact rats and that significant reductions in SND from peak levels were evident during the progressive LeTx-induced hypotension in intact and SAD rats, it is likely that altered regulation of visceral SND regulation contributes, at least in part, to the inability of mammals to regulate arterial blood pressure during *B. anthracis* infection.

An important organizational strategy employed by the sympathetic nervous system to regulate the diverse array of physiological changes required to respond to various challenges is the generation of nonuniform nerve responses. In the present study, we determined the effects of LeTx infusions on the activity in sympathetic nerves innervating three specific target organs (kidney, spleen, and adrenal gland). The response profiles to LeTx infusion for each of the sympathetic nerves were qualitatively similar, suggesting a lack of LeTx-induced sympathetic neural nonuniformity, at least with regard to visceral sympathetic nerve outflow. Although direct recordings of SND provide an output measure of central sympathetic neural circuits, it must be considered that intravenous LeTx infusions may influence SND regulation at ganglionic sites. In the present experiments, pharmacological ganglionic blockade produced by chlorisondamine administration eliminated activity in the renal and splenic nerves, demonstrating that these recordings were primarily from postganglionic fibers. It has been reported that the sympathetic innervation to the adrenal medulla contains preganglionic and postganglionic sympathetic nerves (2). However, in the present experiments, chlorisondamine administration did not substantially affect the level of adrenal SND, indicating that the adrenal nerve recordings contained primarily preganglionic fibers, supporting the idea that LeTx-induced decreases in SND are not mediated exclusively at ganglionic sites.

Consistent with previous results (5, 6), a considerable amount of variability in the absolute duration of LeTx experiments was observed in intact and SAD rats. The present findings do not address factors that may contribute to the interexperiment variability regarding the absolute duration of LeTx infusion experiments; however, normalization of experimental length revealed that the relative occurrence of PK and PD points was similar during LeTx infusions in nonsurviving rats, despite marked variability in the absolute duration of LeTx infusion experiments. As noted previously, two experimental animals in the present study survived the LeTx infusion protocol, consistent with previous LeTx infusion studies (5, 6) that reported a subgroup of surviving animals. Although PK LeTx-induced increases in HR and splenic SND were significantly higher in SAD than intact rats, for the most part, cardiovascular and SND responses to LeTx infusions were similar in intact and SAD rats, suggesting that the arterial baroreceptor reflex did not play an important compensatory role in response to the marked LeTx-induced hypotension. The lack of a compensatory baroreceptor reflex sympathoexcitatory response is not due to an anesthetic effect, as SNP-induced reductions in MAP in anesthetized, intact rats were associated with marked increases in SND. In addition, the lack of reflex-induced increases in visceral SND in response to SNP-induced reductions in MAP in SAD rats demonstrates the efficacy of the SAD procedure in the present experiments.

The present results provide insight regarding SND regulation in response to a specific experimental intervention (LeTx infusion) using an in vivo, anesthetized preparation; thus they cannot be directly applied to other interventions or experimental preparations. Because anesthesia can affect basal levels of sympathetic nerve activity (27) and alter SND responses to various stimuli (24, 25), it must be considered that the present findings may be influenced by the anesthetic state. However, despite this potential limitation, it was important to record the discharges in several sympathetic nerves, because LeTx infusions influence multiple organ systems and elicit numerous physiological responses. The simultaneous recording of discharges in multiple sympathetic nerves is a methodology that, at least in our hands, is typically completed in anesthetized preparations. Moreover, responses to acute physiological stressors can be influenced by behavior modifications; therefore, we chose to study visceral SND regulation in response to continuous LeTx infusions in anesthetized rats to eliminate this influence.

**Perspectives and Significance**

The sympathetic nervous system plays an essential role in maintaining homeostasis in response to acute and chronic physiological stressors. The ability to increase SND in response to various stimuli, such as hypotension, is critical for maintaining adequate tissue perfusion and O2 delivery. *B. anthracis* infection is a lethal pathophysiologival condition associated with marked reductions in arterial blood pressure, and in recent years the amount of research seeking to understand the pathogenesis of *B. anthracis* has intensified. However, mechanisms mediating LeTx-induced hypotension remain unknown. The results of the present study indicate that discharges of anthrax LeTx produce an initial state of cardiovascular and sympathetic nervous system activation, followed by progressive and marked reductions in MAP and visceral SND, in baroreceptor-intact and -denervated rats. These findings demonstrate that the regulation of sympathetic nerve outflow is altered in a complex manner by anthrax LeTx. The present results provide insight into mechanisms contributing to cardiovascular alterations that occur during *B. anthracis* sepsicemia. The elaboration of mechanisms mediating circulatory shock in response to *B. anthracis* infection is important for determining the efficacy of conventional therapy and the development of improved therapies.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

A.A.G., R.J.F., and M.J.K. are responsible for conception and design of the research; A.A.G., R.J.F., and M.J.K. performed the experiments; A.A.G., R.J.F., L.J.M., and M.J.K. analyzed the data; A.A.G., R.J.F., L.J.M., and...
M.J.K. interpreted the results of the experiments; A.A.G., R.J.F., L.J.M., and M.J.K. prepared the figures; A.A.G., R.J.F., L.J.M., and M.J.K. drafted the manuscript; A.A.G., R.J.F., L.J.M., and M.J.K. edited and revised the manuscript; A.A.G., R.J.F., L.J.M., and M.J.K. approved the final version of the manuscript.

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